

A STUDY OF SOME METHODS
FOR THE ANALYSIS OF
ANILINES,
WITH SPECIAL REFERENCE TO
COULOMETRY
AND
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
by
Lars-Åke Truedsson

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LUND INSTITUTE OF TECHNOLOGY
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Title and subtitle A study of some methods for the analysis of anilines, with special reference to coulometry and high performance liquid chromatography				
Abstract A general method is described for the coulometric titration of alkyanilines and of various substituted anilines with anodically generated bromine. The reaction was carried out in water-acetic acid medium and the reactivity was governed by varying the water content and the concentration of bromide ion and by the addition of pyridine. In this way the majority of the anilines tested could be titrated quantitatively. Product formation in the quantitative coulometric bromination of alkyanilines was studied by straight- and reversed-phase liquid chromatography (LC). It was found that the coulometric method yields up to the end-point predictable, unambiguous products by exchange of hydrogen for bromine in free <u>ortho</u> - and <u>para</u> -positions. After the end-point, oxidation products may be formed from prim. anilines and N-alkyl groups split off from sec. and tert. anilines. The retention behaviour of alkyl-substituted prim., sec. and tert. anilines as well as of brominated alkyanilines were investigated using both straight-phase LC on nitrile phase and reversed-phase LC on C ₁₈ and C ₈ phase. On the nitrile phase the elution order is tert., sec. and prim. alkyanilines and within each group the retention is mainly determined by the base strength, the number and size of <u>ortho</u> -situated alkyl groups and by the size of the alkyl groups situated at the nitrogen atom. On the reversed-phase the anilines within each group are mainly eluted after increasing alkyl carbon number. For all brominated anilines investigated, it turned out that an increase in retention generally took place on the introduction of bromine into the aniline nucleus, but for the formation of <u>o</u> -bromoanilines and most <u>para</u> -brominated tert. alkyanilines in straight-phase LC, where retention decreased. A semi-linear relationship was found to exist between capacity factors of brominated and non-brominated anilines in both of the LC systems.				
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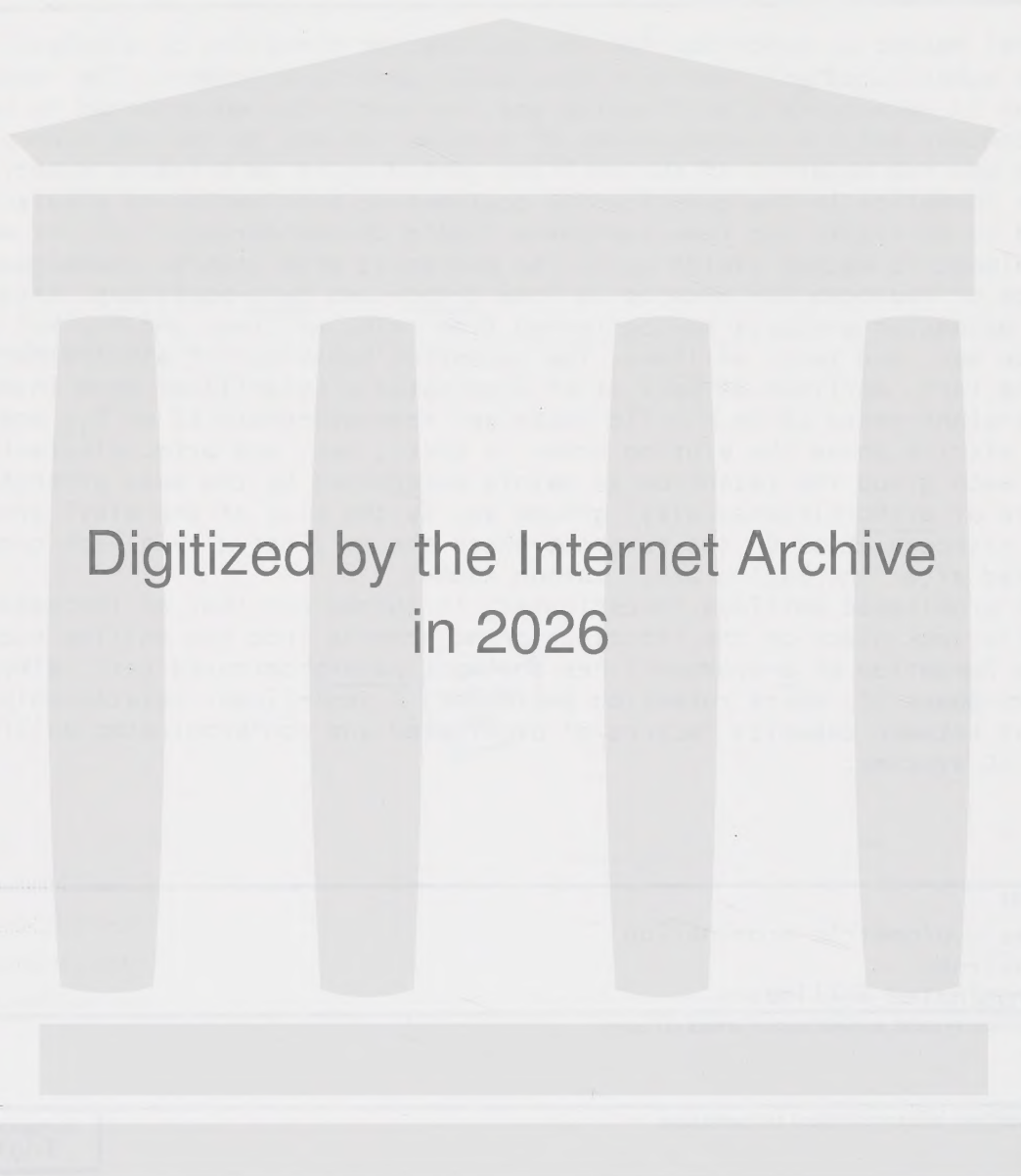
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A STUDY OF SOME METHODS FOR THE ANALYSIS OF ANILINES
WITH SPECIAL REFERENCE TO
COULOMETRY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

This review summarizes and discusses results from the following papers which will be referred to by Roman letters I-V.

- I L.-Å. Truedsson and B.E.F. Smith. A General Method for Coulometric Titration of Alkylanilines with Bromine. *Talanta*, 26 (1979) 487.
- II L.-Å. Truedsson. Coulometric Titration of Anilines, mainly Containing Deactivating Functional Groups, with Bromine. *Talanta* 26 (1979) 493.
- III L.-Å. Truedsson and B.E.F. Smith. Study of Retention Behaviour of Primary, Secondary and Tertiary Anilines in Straight- and Reversed-Phase Liquid Chromatography. *J. Chromatogr.*, submitted for publication.
- IV L.-Å. Truedsson and B.E.F. Smith. Liquid Chromatography Study of Brominated Anilines and Investigation of Product Formation in the Bromination Reaction. I. Anilines without Ring Substituents or with Alkyl Groups in the Ortho- and Para-Positions. *J. Chromatogr.*, submitted for publication.
- V L.-Å. Truedsson. Liquid Chromatography Study of Brominated Anilines and Investigation of Product Formation in the Bromination Reaction. II. Anilines with Alkyl Groups in the Meta-Position. *J. Chromatogr.*, submitted for publication.

INTRODUCTION

Industrial use of anilines

Aniline and its derivatives are commercially important compounds which are used as starting materials for the preparation of a large number of substances having a wide variety of applications. Among the major uses of anilines are for the preparation of vulcanization accelerators, antioxidants and antidegradants in the rubber industry¹.

Anilines are also employed in the production of epoxy resins and find wide use as additives in the petroleum industry and as dye intermediates in the textile industry. A major use of anilines is as intermediates for the preparation of compounds that are toxic to bacteria, fungi, herbs, insects, mollusks, nematodes, weeds, and other pests. In the pharmaceutical industry, the most important use of aniline derivatives is for the preparation of synthetic sweetening agents, sulpha drugs and acetanilide (antifebrin)¹.

Methods of analysis

Aniline and many of its derivatives are susceptible to oxidation with a corresponding darkening in colour. Thus, the colour of the sample to be analyzed can be used as a crude measure of its purity¹.

Numerous solid derivatives of the various anilines are known and can be used for identification and purification purposes. Diazotization and coupling with R-salt has been employed as a rapid method for the identification of aniline and some of its derivatives. This can also be accomplished by the production of various colours upon treatment with certain reagents, *e.g.* ninhydrin and ammonium ceric nitrate, as well as by means of spectroscopic analyses, *e.g.* ultra-violet and infrared¹.

Because of the basic properties of the amine function in aniline and most of its derivatives, a simple acid-base type of titration can often be employed for their quantitative determination. The most useful methods for such weak bases are based on nonaqueous titration

with perchloric acid in glacial acetic acid². Other titrimetric methods are based on the reaction between free anilines and bromine.³⁻⁵ Primary anilines can also be quantitatively determined by diazotization with standardized sodium nitrite solution in the presence of hydrochloric acid⁶.

The separation of anilines from other substances present, by gas¹ and liquid chromatography⁷, followed by measurement of the aniline eluted, can be utilized as an assay method. The methods studied in this work are a titrimetric method and a liquid chromatography (LC) separation method. Thus, "Coulometric titration of anilines with bromine" (I, II) belong to the former and "Chromatographic studies of alkyanilines and brominated alkyanilines" (III, IV and V) belong to the latter group.

COULOMETRIC TITRATION OF ANILINES WITH BROMINE (I, II)

This first part of the thesis includes two papers. The first paper deals with the coulometric titration of alkyanilines with bromine (I) and the second paper with the coulometric titration of mostly deactivated anilines with bromine (II).

C o u l o m e t r i c a n a l y s i s

Coulometric analysis is a designation of an analytical technique in which the quantity of electricity is employed to determine the amount of chemical substance in solution.

Coulometric analysis can be performed in two different ways. In the technique used in this thesis, *i.e.* coulometric titration, a constant current is used for the production of a reagent and is passed until an indicator signals completion of the reaction. The quantity of electricity passed to reach the end-point is calculated from the magnitude of the current and the time required. In the second technique the potential of the working electrode is maintained at some predetermined value, dependent on the required reaction, and the completion of the analysis is indicated when the current flow reaches zero. Here coulometric analysis has been used

for the titration of alkyanilines and deactivated anilines with bromine, coulometrically generated. The equipment used is assembled from inexpensive standard pieces of apparatus thus making the method generally accessible.

Fig. 1 shows the coulometric cell used in (I) and (II) and Fig. 2 a typical titration curve obtained in the coulometric titration.

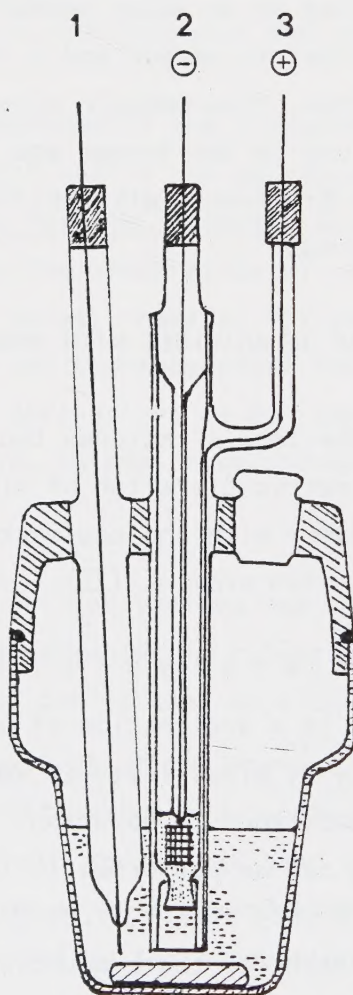


Fig. 1. Cell for coulometric titration. 1 = indicating electrode; 2 = cathode containing 2 mole l^{-1} sulphuric acid; 3 = generating electrode (anode)

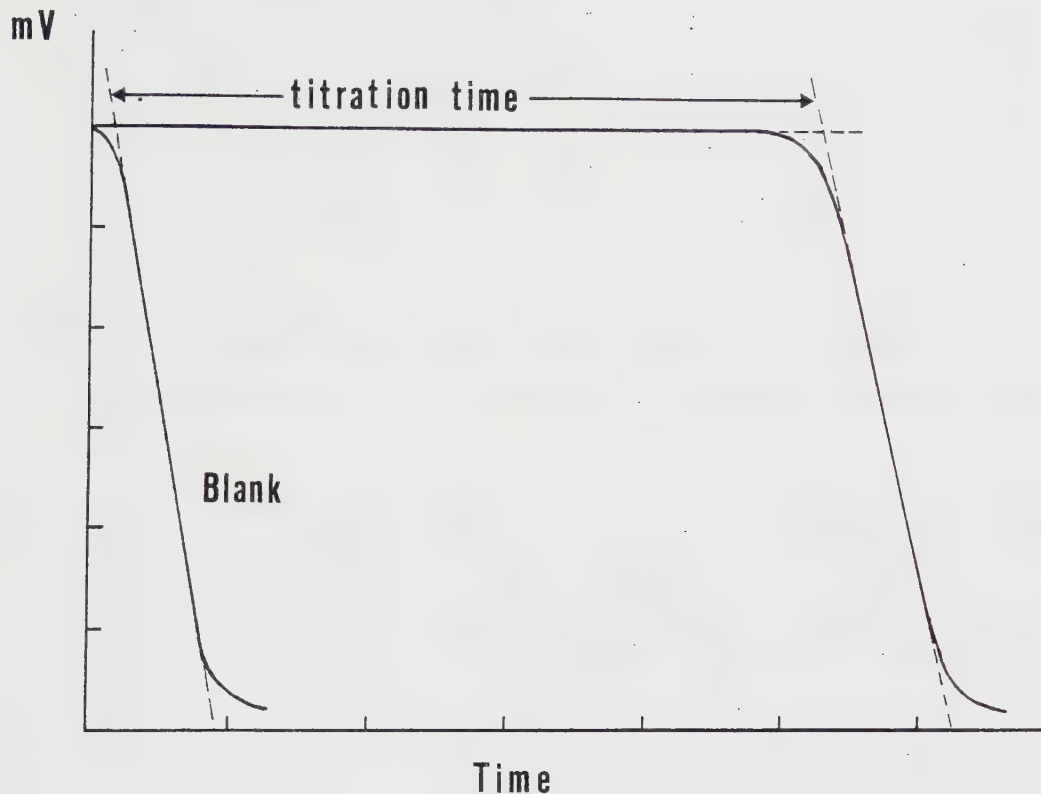


Fig. 2. Titration curves and method for evaluation of the end-point

Titration procedure. The titration procedure was as follows. An amount of the aniline calculated to consume 10-20 μeq of bromine was dispensed into the titration vessel in acetic acid solution by means of an Agla micrometer syringe. Twenty mls of the titration medium were added and the electrodes inserted and connected to the electric circuits. The magnetic stirrer was set at a relatively high speed and the pH-meter set to READ. The generator current was adjusted to 3,000 mA, the recorder speed to 30 mm/min and the measuring range set to 1 V.

The bromine generation was initiated simultaneously as the recorder pen crossed a vertical line on the chart after a stable base-line had been achieved. The generation of bromine was continued

until depolarization of the indicator electrode was complete. The progress of the titration as well as the method for determining the equivalence point are shown in Fig. 2. A blank was titrated in the same way and the amount of bromine consumed by the aniline is equivalent to the "titration time" in Fig. 2.

To calculate the amount of an analyte, Faraday's law is used. This law states that "The amount of chemical change produced by an electric current is proportional to the quantity of electricity passed". If 100 per cent current efficiency is obtained, then Faraday's law can be expressed by the following equation. For a constant current

$$m = \frac{t \cdot I \cdot E \cdot 1000}{F} \quad (1)$$

m = the weight of substance in μg

E = equivalent weight of substance

I = generating current in mA

t = titration time in seconds

F = Faraday's constant = 96487 C/eq

Influence of various factors on the bromination reaction and on the shape of the titration curve

Structural influence on the bromination of anilines. The reaction between anilines and the electrophilic reagent bromine is the basis of the quantitative titration of anilines with this reagent. The dominating influence in the bromination of anilines is exerted by the amino group, whose well-known activation of the aromatic ring towards electrophilic reagents causes bromine to replace hydrogen at the free *ortho*- and *para*-positions. Other substituents in the aniline only have a modifying influence on the reaction. Thus, prim. alkylanilines generally are more reactive than aniline because of the electron releasing effect of alkyl groups towards the ring. Anilines with deactivating substituents, e.g. halo, acetyl, formyl and carboxyl groups show no great difference in reactivity from anilines with activating substitu-

ents, *e.g.* alkyl and alkoxy, when substituted in the 2- and 4-position relative to the amino group. However, when the substituent group is situated in the 3-position, the deactivating substituents cause the reactivity to diminish. For anilines with strongly deactivating substituents like nitro groups, the difference in reactivity can be appreciable. The steric surroundings of the nitrogen atom is also important for the outcome of the bromination reaction. Sec. and especially tert. anilines are *e.g.* less reactive than corresponding prim. anilines. The choice of titration medium is also of decisive importance for the result for some anilines.

Choice of titration medium. The coulometric generation of the reagent bromine instead of delivering it, or bromide-bromate, from a byrette as usually done, means a great step forward towards a general quantitative bromination method for different kinds of anilines. It has been shown that acetic acid-water is a very good titration medium for volumetric bromination of phenols⁸, which are structurally analogous to anilines, permitting the quantitative bromination of a great number of phenols with good accuracy. However, in order to get quantitative results the titration conditions had to be adapted to the type of phenol and accordingly frequently changed. Later on, a general method for coulometric titration of phenols in aqueous acetic acid was developed⁹.

The phenol bromination method introduced by Koppeschaar¹⁰ as early as at the end of the previous century, using free bromine as a titrant has, with certain modifications been used also for the analysis of various anilines^{3,4}. In order to develop a general coulometric bromination method for anilines, the present study was undertaken and the usefulness of aqueous acetic acid as a titration medium especially examined.

The reactivity-promoting properties of the titration medium were — governed by (i) the ratio of acetic acid to water, (ii) the concentration of bromide ion and (iii) the presence of pyridine (see Table I). With proper choice of titration medium both activated and deactivated anilines can be titrated quantitatively with a few exceptions (see Tables II and IV).

Table I. Composition of media used for bromination of anilines

Medium	Acetic acid % v/v	Water % v/v	Pyridine % v/v	[Br ⁻] mole l ⁻¹
I-1	55	35	10	0.1
I-2	55	35	10	0.4
I-3	55	35	10	1.2
II-3	55	40	5	1.2
III-1	60	40	-	0.1

Generating current. Ideally the generating current should have a value that corresponds to the bromine consumption in the proper substitution reaction. In that way side-reactions due to bromine excess, such as oxidation and bromine substitution in side chains are avoided. On the other hand, at low currents the analysis time will be too long and side-reactions of an unknown nature can cause erroneous results. A current of 3 mA was found suitable when determining anilines in amounts in the range 300-2000 μ g.

Amount of aniline. The amount of aniline titrated is not insignificant. There is in fact a lower as well as an upper limit. The lower limit is set partly by the fact that a certain minimum distance is needed on the recorder chart in order to get an accurate measure of time (see Fig. 2). The lower limit is also influenced by a lack of correspondence between the rates of bromine generation and of consumption.

The upper limit for the amount of aniline is partly determined by certain side-reactions at the generator anode and partly by a contamination of the indicator electrode. These reactions seem to start after a certain time of electrolysis and cause a relative error which increases with the analysis time, *i.e.* with the amount of aniline. The upper limit has not been settled, and seems also to be dependent on the kind of aniline titrated. However, it is recommended that the amount of aniline titrated should consume between 10 and 20 μ eq. of bromine.

Polarizing resistance. The titration was followed by means of an indicating double platinum point electrode, connected to the polarizing voltage terminal of a pH-meter through a polarizing resistance¹¹. The magnitude of this resistance has a decisive influence on the appearance of the titration curve. A resistance of 100 k Ω was found to give the most rapid depolarization, thus permitting an accurate evaluation of the end-point. It should be noted, that the size of the polarizing resistance (100 k Ω) should be adapted to the magnitude of the bromine generating current (3 mA).

R e s u l t s a n d d i s c u s s i o n

Alkylanilines (I)

Forty alkylanilines have been investigated by the coulometric titration method. Of these, 17 are prim. anilines, 12 secondary and 11 tert. anilines. With the exceptions of N,N-dimethyl-2,6-dimethylaniline, N,N-diethyl-2-methylaniline and N,N-diethyl-2-ethylaniline, all could be titrated quantitatively (see Tables II and III). However, the mentioned tert. *ortho*- or di-*ortho*-substituted anilines did consume some bromine, but only slowly, and an excess of bromine was necessary for the reaction to take place.

Prim. anilines. All the prim. alkylanilines investigated could be titrated in the pyridine-free medium III-1, containing 60% (v/v) acetic acid and being 0.1 mole l⁻¹ with respect to bromide (see Table II). In this medium all *ortho*-*para*-standing hydrogen atoms were substituted with bromine. The reaction between aniline and bromine is somewhat sluggish in medium III-1, which is shown by the reduced slope of titration curve. Accordingly, low results were obtained. On that account, the pyridine-containing media I-1 or II-3, where a more rapid depolarization generally occurs, are recommended.

It was also found that the bromide content had a greater influence on the reactivity of aniline than the presence or absence of pyridine, as only 2 moles of bromine per mole of aniline were consumed in the pyridine-containing medium II-3 compared with the 3 moles in the pyridine-free medium III-1. The bromide content in II-3 is 1.2 mole l⁻¹ compared to 0.1 mole l⁻¹ in III-1.

Table II. Media for quantitative bromination of alkyanilines

Type of aniline	Medium	Number of available hydrogen atoms exchanged for bromine
Primary alkyanilines		
With and without substituents	III-1 or I	All
	II-3*	2 of 3
Secondary alkyanilines		
Without nuclear substituents	III-1 or I	2 of 3
With nuclear substituents in 3-position	III-1	2 of 3
	I-1	All (3)
in 2- or 4-position	I-1	All (2)
Tertiary alkyanilines		
Without nuclear substituents	III-1	1 of 3
	I	2 or 1 of 3
With nuclear substituents in 3-position	III-1	1 of 3
	I-1	2 of 3
in 2- or 4-position	I	1 of 2 or none

*Aniline

2,4,6-Trimethylaniline does not have any free *ortho*- and *para*-positions, but consumes nevertheless one mole of bromine per mole of aniline in the pyridine-containing media. This reaction is thought to be analogous to the reaction between 2,4,6-trimethylphenol and bromine, where a *para*-quinoid bromocyclohexadienone is formed⁹.

Sec. anilines have a decreased reactivity towards bromine in comparison with prim. anilines in the pyridine-free medium III-1. This is sometimes shown by a sloping titration curve or in a decreased consumption of bromine. In the recommended pyridine-containing media, the anilines with nuclear alkyl groups were fully brominated, while anilines without nuclear alkyl groups were only dibrominated.

Table III. Results of bromination of alkyylanilines

Aniline	Titn. medium	Number of H substituted	Mean error, %	Titn. medium	Number of H substituted	Mean error, %
C ₆ H ₅ NH ₂	III-1	3	-4.5	I-1	3	-1.6
	—	—	—	II-3	2	-0.8
C ₆ H ₅ NH ₂ , HCl	III-1	3	-4.0	I-1	3	-2.5
2-Me	III-1	2	+0.4			
2-Et	III-1	2	-1.0			
2-isoPr	III-1	2	-0.8			
3-Me	III-1	3	+0.1	I-1	3	-0.5
3-Et	III-1	3	-0.1			
3-Et, HCl	III-1	3	-0.9			
4-Me	III-1	2	-0.3	I-1	2	+0.4
4-Et	III-1	2	+0.8			
4-Et, HCl	III-1	2	-0.9			
4-isoPr	III-1	2	-0.8			
4-Bu	III-1	2	-0.2	I-1	2	-0.2
2,3-diMe	III-1	2	-0.4			
2,4-diMe	III-1	1	+1.1			
2-Me-4-Bu	III-1	1	-2.5	I-3	1	-1.6
2,5-diMe	III-1	2	-0.7			
2,6-diMe	III-1	1	+0.8			
3,4-diMe	III-1	2	+0.5			
2,4,6-triMe	III-1	0	—	I-1	1	+3.4
N-Me	III-1	2	-0.6	I-2	2	-0.6
N-Et	III-1	2	-1.4	I-2	2	-0.8
N-Pr	III-1	2	-1.3	I-3	2	-0.8
N-Bu	III-1	2	-1.3	I-2	2	-1.0
N-Bz	III-1	2	-2.8	I-1	2	-1.9
N-Ph	III-1	4	-3.7	I-1	4	-3.0
N-Me-2-Me	III-1	2	*	I-1	2	-0.6
N-Et-2-Me	III-1	1	+2.1	I-1	2	+0.1
N-Me-4-Me	III-1	2	*	I-1	2	+1.0
N-Et-4-Me	III-1	0	—	I-1	2	-0.2
N-Et-3-Me	III-1	2	-0.6	I-1	3	-1.4
N-Me-3-Me	III-1	2	+0.8	I-1	3	-2.8
				I-3	2	-1.3
N,N-diMe	III-1	1	+1.0	I-1	2	±0.0
N,N-diEt	III-1	1	-1.3	I-3	1	+2.3
N,N-diPh	III-1	3	-0.6	I-1	3	-0.2
N,N-diMe-2-Me	III-1	0	—	I-2	1	+2.8
N,N-diEt-2-Me	III-1	0	—	I-1	0	—
N,N-diEt-2-Et	III-1	0	—	I-1	0	—
N,N-diMe-3-Me	III-1	1	-0.7	I-1	2	-0.6
N,N-diEt-3-Me	III-1	1	-1.2	I-1	2	-3.7
N,N-diMe-4-Me	III-1	0	—	I-1	1	+3.6
N,N-diEt-4-Me	III-1	0	—	I-1	1	+0.1

* Sloping titration curve, difficult to evaluate.

Tert. anilines show a further decreased reactivity towards bromine in comparison with *sec. anilines*, and were not fully brominated even in the reactivity-promoting pyridine-containing media. In fact, the *ortho*-alkyl-substituted *tert. anilines* did give very sloping titration curves already from the start of the titration. The decreased reactivity of the *sec.* and *tert. anilines* are thought to be due to steric interactions between the alkyl groups on the nitrogen atom and the *ortho*-situated substituents, (alkyl groups and/or bromine atoms).

Anilines with alkyl groups in the *meta*-position, on the other hand, were generally more reactive with bromine than other types of alkyl-anilines, due to the co-operation of the *ortho-para*-directing influences of the amino group and the *meta*-standing alkyl groups. The mean relative error, for the best medium for each compound, was $\pm 0.9\%$ for the prim. and sec. anilines and $\pm 1.2\%$ for the tertiary.

Haloanilines and anilines containing various oxygenated functional groups (II)

Fortynine substituted anilines have been investigated by the coulometric bromination method. Of these were 26 halogenated anilines. The other substituted anilines include compounds with the following substituents: methoxy, formyl, acetyl, carboxyl, ester, carboxymethyl (CH_2COOH), nitro and sulphonate. The anilines substituted with the oxygenated groups were on the whole more difficult to assay than the haloanilines and gave less accurate results. The mean relative error for the best medium was $\pm 0.5\%$ for the latter and ± 1.0 for the former, (see Tables IV, V and VI).

Only two tert. anilines and no sec. anilines were available for the investigation. Nearly all of the anilines could be titrated in both the pyridine-free and pyridine-containing media, but the latter are generally recommended, because of the better results obtained, partly due to the fact that the titration curve was better defined in the pyridine-containing media. Strongly deactivated anilines, *i.e.* trichloroanilines, *m*-nitroaniline and the dinitroanilines failed to give titration curves usable for quantitative determination. In fact, the dinitroanilines gave a titration curve that was identical to the blank, whereas the others gave sloping curves. *p*-Iodoaniline could not be determined quantitatively due to a fast contamination of the indicating electrode.

Anilines are sensitive to oxidation, and as bromine is an oxidant, it can oxidize some compounds or displace substituent groups such as formyl, carboxyl and sulphonic acid groups, thus causing overconsumption of bromine. Delgado⁵ has demonstrated that the decarboxylation of *p*-aminobenzoic acid on bromination in aqueous media is a function of pH and increases when the pH is increased, becoming quantitative at pH 5. No displacement of carboxyl groups in the prim. *o*- and *p*-carboxyanilines took place in the pyridine-free medium III-1, which is therefore recommended.

Table IV. Media for quantitative bromination of anilines mainly containing deactivating functional groups

Functional group	Medium	Number of available hydrogen atoms exchanged for bromine
Halogen (2- or 4-)		
F	I-1	All
Cl	I-1	All
Br	I-3	1 of 2
I (2-)	III-1	All
Halogen (3-)		
F	I-3	2 of 3
Cl	I-3 (III-1)	2 of 3
Br	I-3	2 of 3
Halogen (di-)		
Cl (2,3-)	I-3	1 of 2
F (2,4-)	I-1	All
Cl (2,4-)	I-1	All
Br (2,4-)	III-1	All
F (2,5-)	I-3	1 of 2
Cl (2,5-)	III-1 (I-3)	1 of 2
Cl (2,6-)	III-1 (I-1)	All
Cl (3,4-)	I-3	1 of 2
Cl (3,5-)	III-1 (I-1)	2 of 3
Methyl-chlorine		
2-Cl-6-Me	I-1 (III-1)	All
3-Cl-2-Me	I-1 (III-1)	All
3-Cl-4-Me	I-1 (III-1)	All
5-Cl-2-Me	I-1 (III-1)	All
Methoxy (2-)		
Methoxy (4-)	III-1	All
Formyl (2-)	III-1	1 of 2*
Formyl (3-)	I-2	2 of 3
N,N-Me ₂ -4-Formyl	I-1	1 of 2
4-Acetyl	I-3	1 of 2
Carboxy (2-)	III-1	All
Carboxyl (3-)	I-1	All
Carboxyl (4-)	III-1	All
Ester (2-)	I-3	1 of 2
Ester (4-)	I-3	1 of 2
Carboxymethyl (4-)	I-1	All
Sulphonic (2-)	I-1 (III-1)	All
Sulphonic (3-)	I-1	2 of 3
Sulphonic (4-)	III-1 (I-1)	All
Nitro (2-)	I-1	1 of 2
Nitro (4-)	I-1 (III-1)	1 of 2

*Further bromine consumption after the break used for determination of end-point

Table V. Results of bromination of haloanilines

Aniline	Titn. medium	Number of H substituted	Number of breaks in the titration curve	Mean relative error, %	Titn. medium	Number of H substituted	Number of breaks in the titration curve	Mean relative error, %
2-Fluoro	I-1	2	1	-0.8	III-1	2	1	-2.6
3-Fluoro	I-3	2	1	-0.5	III-1	2	1	+2.3
4-Fluoro	I-1	2	1	-0.2	III-1	1-2	1	†
2,4-Difluoro	I-1	1	1	-0.6	III-1	~1	1	†
2,5-Difluoro	I-3	1	1	+0.4	III-1	1	1§	+1.7
2-Chloro	I-1	2	2(2)	+0.3	III-1	2	2(2)	-1.5
2-Chloro-6-methyl	I-1	1	1	+0.2	III-1	1	1	+0.3
3-Chloro	I-3	2	1	-0.3	III-1	2	2(1)§	+0.3
3-Chloro-2-methyl	I-1	2	1	-0.6	III-1	2	1	-0.5
3-Chloro-4-methyl	I-1	2	1	-0.7	III-1	2	1	-1.3
5-Chloro-2-methyl	I-1	2	1	+0.4	III-1	2	1	-0.3
4-Chloro	I-1	2	2(2)	-0.8	III-1	~2	1	†
2,3-Dichloro	I-3	1	1§	+0.4	III-1	~1	2(1)§	†
2,4-Dichloro	I-1	1	1	+0.1	III-1	~1	1	†
2,5-Dichloro	I-3	1	1	-0.7	III-1	1	2(1)§	+0.4
2,6-Dichloro	I-1	1	1	+1.0	III-1	1	1	-0.7
3,4-Dichloro	I-3	1	1	+1.0	III-1	~1	2(1)§	†
3,5-Dichloro	I-1	2	1§	+0.6	III-1	2	1	+0.1
2,3,4-Trichloro	I-1	~1	1	†	III-1	~1	1	†
2,4,5-Trichloro	I-1	~1	1	†	III-1	~1	1	†
2-Bromo	I-1	2	2(2)	-2.6	III-1	~2	2(2)	†
	I-3	1	1§	+0.2				
3-Bromo	I-3	2	1	+0.3	III-1	~2	2(1)§	†
4-Bromo	I-1	2	2(2)	-2.5	III-1	~2	1	†
	I-3	1	1§	-0.3				
2,4-Dibromo	I-1	1	1	+1.4	III-1	1	1	-0.3
2-Iodo	I-1	2	2(2)	+0.9	III-1	2	2(2)	+0.2
4-Iodo	I-1	~2	2(2)	†	III-1	~2	2(2)	†

† Mean relative error greater than 3%.

§ Further bromine consumption after the break used for determination of end-point.

Table VI. Results of bromination of anilines containing various oxygenated functional groups

Aniline	Titn. medium	Number of H substituted	Number of breaks in the titration curve	Mean relative error, %	Titn. medium	Number of H substituted	Number of breaks in the titration curve	Mean relative error, %
2-Methoxy	I-2	2	1	±0.0	III-1	2	1	+1.0
4-Methoxy	I-media	†	†	†	III-1	2	1	+0.8
2-Formyl	I-3	~1	1†	§	III-1	1	2(1)†	+1.5
3-Formyl	I-2	2	2(2)	-0.5	III-1	~2	1	§
N,N-Dimethyl-4-formyl	I-1	1	1	+1.7	III-1	~1	1	§
4-Acetyl	I-3	1	1	+1.5	III-1	~2	2(2)	§
4,4'-Bisdimethylaminobenzo-phenone	I-1	2	1	+1.3	III-1	1-2	1	§
2-Carboxy	I-3	2	2(2)	+0.9	III-1	2	1	-0.1
3-Carboxy	I-1	3	1	-1.0	III-1	~2	1	§
N,N-Dimethyl-3-carboxy	I-1	~1	1	§	III-1	1	1	±1.4
4-Carboxy	I-1	2-3	2(2)	§	III-1	2	2(2)	±0.6
2-Carboethoxy	I-3	1	1†	+1.5	III-1	~2	2(2)	§
4-Carboethoxy	I-3	1	1†	+0.8	III-1	1-2	2(2)	§
4-Carboxy-methyl	I-1	2	1	-1.7	III-1	~2	1	§
2-Sulphonic acid	I-1	2	1	-0.5	III-1	2	1	-0.5
3-Sulphonic acid	I-1	2	1	-1.7	III-1	~2	1	§
4-Sulphonic acid	I-1	2	2(2)	-0.3	III-1	2	1	-0.1
2-Nitro	I-1	1	1	-0.9	III-1	~1	1	§
3-Nitro	I-2	~1	1†	§	III-1	~1	1	§
4-Nitro	I-1	1	1	-0.4	III-1	1	1	-0.4
Dinitro-(2,4-,2,6-,3,5-)	I-media	0	0	—	III-1	0	0	—

† Not possible to determine from the titration curve.

§ Mean relative error greater than 3%.

† Further bromine consumption after the break used for determination of end-point.

The presence of the two methyl groups at the nitrogen atom in N,N-dimethyl-3-carboxyaniline decreases the number of entering bromine atoms as compared with the corresponding prim. aniline. This is in accordance with the results previously found for the alkyanilines. The methoxyanilines differ from the other compounds with oxygenated functional groups in having an activating functional group in addition to the amino group. Thus, both *ortho*- and *para*-methoxy-substituted anilines were fully brominated in the pyridine-free medium. However, in the more reactive pyridine-containing media the *p*-methoxyaniline could not be evaluated because of oxidation, as indicated by a change of colour.

ANALYSIS OF ANILINES AND BROMINATED ANILINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) (III-V)

The retention behaviour of prim., sec. and tert. alkyanilines (III) as well as of brominated alkyanilines (IV, V) was studied, using straight- and reversed-phase liquid chromatography (LC). In paper (III) two reversed-phase systems on octadecylsilane (C_{18}) and octylsilane (C_8), respectively, and one straight-phase system on nitrile phase have been used. In papers (IV) and (V), where also product formation in the coulometric bromination of alkyanilines was investigated, the same straight-phase system as above was employed, while only the C_{18} phase was used for the reversed-phase study.

Alkyanilines (III)

Retention behaviour of alkyanilines

The retention behaviour of a series of alkyl-substituted prim., sec. and tert. anilines was studied on three liquid chromatographic systems: one straight-phase system on nitrile phase and two reversed-phase systems on C_{18} and C_8 phase, respectively (see Table VII). On the straight-phase system, the elution order is tert., sec. and prim. anilines and within each group the retention is mainly determined by the base strength, the number and size of *ortho*-situated alkyl groups and by the size of the alkyl groups substituted at the nitrogen atom (see Fig. 3).

Table VII. Capacity factors, k' , for alkyanilines in four LC systems

Straight-phase system: Nucleosil CN. Mobile phase: 0.2% (v/v) 2-propanol in isooctane. Reversed-phase systems: (a) Nucleosil C₁₈. Mobile phases: methanol-aqueous buffer (80:20 and 70:30, v/v, pH = 7.0). (b) LiChrosorb RP8. Mobile phase: methanol-aqueous buffer (60:40, v/v, pH = 7.0)

No.	Aniline (Substituent)	k' Nitrile phase	C_{18} phase		C_8 phase
			Eluent (80:20)	Eluent (70:30)	Eluent (60:40)
Primary anilines					
1	None	7.53	0.41	0.55	0.60
2	2-Methyl	4.84	0.55	0.85	0.92
3	2-Ethyl	3.65	0.71	1.20	1.40
4	2-Isopropyl	3.14	0.86	1.63	2.07
5	3-Methyl	8.07	0.51	0.83	0.94
6	3-Ethyl	7.02	0.67	1.20	1.49
7	4-Methyl	9.62	0.54	0.85	0.97
8	4-Ethyl	8.72	0.71	1.26	1.59
9	4-Isopropyl	8.87	0.90	1.75	2.46
10	4-n-Butyl	8.72	1.38	3.11	4.84
11	2,3-Dimethyl	5.48	0.74	1.18	1.31
12	2,4-Dimethyl	6.04	0.78	1.25	1.49
13	2-Methyl-4-n-butyl	5.07	1.91	4.71	7.40
14	2,5-Dimethyl	4.45	0.75	1.26	1.43
15	2,6-Dimethyl	2.83	0.78	1.31	1.43
16	3,4-Dimethyl	9.51	0.69	1.16	1.40
17	2,4,6-Trimethyl	2.96	1.08	1.99	2.34
Secondary anilines					
18	N-Methyl	2.32	0.68	1.12	1.22
19	N-Methyl-2-methyl	1.43	0.97	1.68	1.86
20	N-Methyl-3-methyl	2.16	0.89	1.62	1.86
21	N-Methyl-4-methyl	2.73	0.90	1.63	1.93
22	N-Ethyl	1.49	0.87	1.54	1.77
23	N-Ethyl-2-methyl	0.82	1.34	2.57	2.91
24	N-Ethyl-3-methyl	1.40	1.14	2.26	2.71
25	N-Ethyl-4-methyl	1.72	1.15	2.26	2.73
26	N-Ethyl-2-ethyl	0.67	1.86	3.80	4.61
27	N-Acetyl		0.41	0.62	0.71
28	N-Acetyl-4-methyl		0.54	0.87	1.09
29	N-Propyl	1.06	1.23	2.35	3.00
30	N-n-Butyl	0.90	1.69	3.70	5.04
31	N-Phenyl	2.50	1.52	3.45	4.86
32	N-Benzyl	1.65	1.50	3.42	4.79
Tertiary anilines					
33	N,N-Dimethyl	0.65	1.35	2.42	2.68
34	N,N-Dimethyl-2-methyl	0.45	1.76	3.48	3.99
35	N,N-Dimethyl-3-methyl	0.68	1.82	3.61	4.17
36	N,N-Dimethyl-4-methyl	0.79	1.80	3.64	4.17
37	N,N-Dimethyl-2,6- -dimethyl	0.14	4.20	10.1	
38	N,N-Diethyl	0.50	2.32	5.21	6.44
39	N,N-Diethyl-2-methyl	0.29	3.45	7.71	8.89
40	N,N-Diethyl-3-methyl	0.54	3.08	7.31	9.18
41	N,N-Diethyl-4-methyl	0.90	2.95	6.81	8.06
42	N,N-Diethyl-2-ethyl	0.21	5.21	12.8	16.8
43	N,N-Diphenyl	0.39	7.40	25.9	

Thus, the ease of access of the nitrogen atom is a dominating factor for the retention¹². The difference in retention between *para*-substituted anilines without *ortho*-substituents and the corresponding *meta*-substituted anilines can be ascribed to the fact that anilines belonging to the former group are stronger bases and accordingly more ionized in the eluent than the members in the latter group. It is also found that there is a semi-linear correlation between pK_b and $\lg k'$ for the prim. and sec. anilines, respectively.

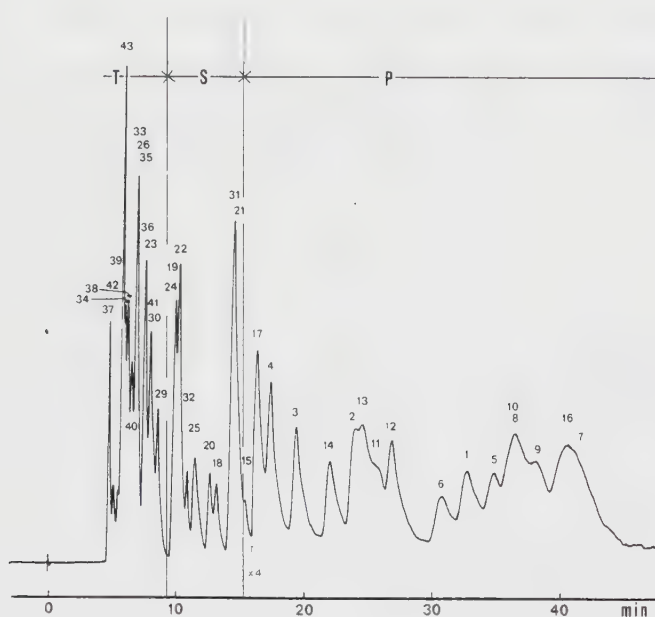


Fig. 3. Class separation of a mixture of prim., sec. and tert. alkylanilines on the nitrile phase. Stationary phase, Nucleosil CN; mobile phase, 0.2% (v/v) 2-propanol in isooctane; $45 \text{ ml} \cdot \text{h}^{-1}$. P = prim. anilines; S = sec. anilines; T = tert. anilines. The numbers refer to Table VII

On the reversed-phases the anilines within each group are mainly eluted after increasing total alkyl carbon number. Sec. anilines have higher retention than primary ones with the same total alkyl carbon number, and the same applies to tert. anilines vs. secondary.

The behaviour of the alkylanilines on the C_8 phase were much as on the C_{18} phase.

Combination of straight- and reversed-phase LC for the separation and identification of alkyylanilines

Identification by two-phase plot. In Fig. 4, $\lg k'$ for the C_{18} phase is plotted against $\lg k'$ for the nitrile phase. The anilines investigated, can be divided into zones containing prim., sec. and tert. anilines, respectively. Although the boundaries between the zones are rather narrow and some mixing of compounds occur, it will be possible to distinguish to a great extent between prim., sec. and tert. alkyl-anilines, zones P, S and T, respectively, and also between di-*ortho*-, mono-*ortho*- and non-*ortho*-alkyl-substituted prim. anilines. In addition, the zone diagram gives information about alkyl carbon number, as anilines with the lowest number appear at the bottom of each zone and those with the highest number at the top.

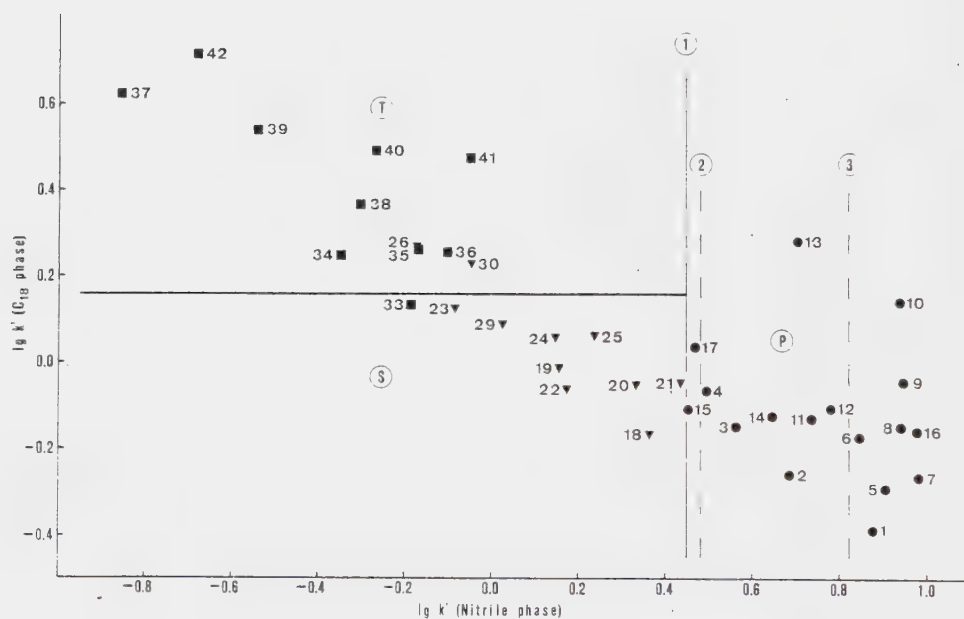


Fig. 4. Two-phase plot of $\lg k'$ of alkyylanilines on reversed-phase [Nucleosil C_{18} , methanol-aqueous buffer (80:20, v/v, pH = 7.0); $45 \text{ ml} \cdot \text{h}^{-1}$] versus $\lg k'$ on straight-phase [Nucleosil CN, 0.2% (v/v) 2-propanol in isooctane; $45 \text{ ml} \cdot \text{h}^{-1}$]. ● and P = prim. anilines; ▼ and S = sec. anilines; ■ and T = tert. anilines. The numbers refer to Table VII

However, because of the mixing of some compounds, *viz.* sec. and tert. anilines, it is impossible to decide whether an aniline that falls in the T-zone is in fact a secondary one. A simple test to

distinguish between the sec. and tert. anilines is to heat the sample for a couple of minutes with an excess of acetic anhydride and then run the product on the nitrile phase system. If no change of retention is observed, the aniline is a tert. aniline as only prim. and sec. anilines react with the acetic anhydride. On the other hand, if no peak is obtained or the retention is much increased, the aniline that did fall into the T-zone was a secondary one. The reason for this behaviour is, that N-acetylated anilines travel very slowly on the nitrile column.

Systematic separation of alkylanilines. The prim. anilines can be separated as a class from sec. and tert. anilines on the nitrile phase and the latter two classes are largely resolved on the same phase (see Fig. 3). If these classes are then run on the C_{18} phase, a separation according to alkyl carbon number is achieved (see Fig. 5). The peaks representing the different alkyl carbon numbers are collected from the C_{18} phase and rerun on the nitrile phase, where they are largely resolved into individual compounds as shown in Fig. 6, where the peaks representing alkyl carbon number 2 of the prim. anilines are separated.

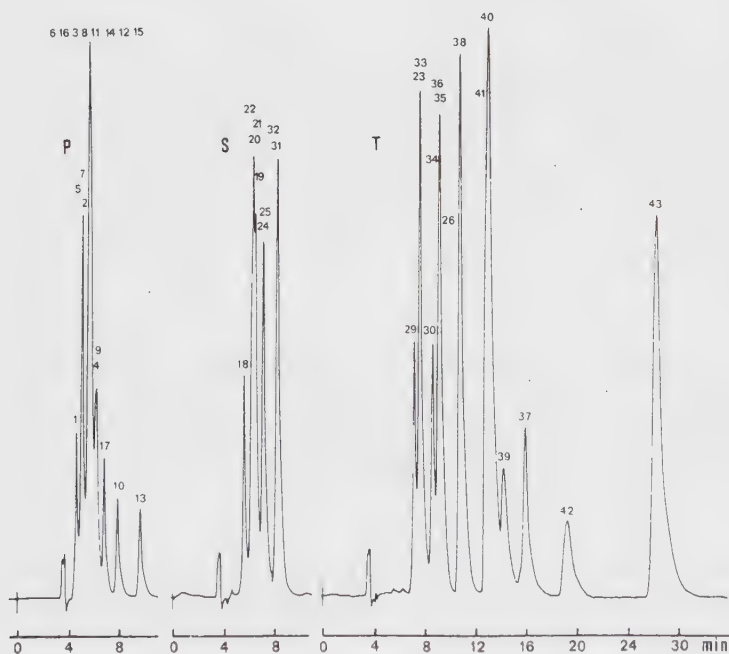


Fig. 5. Carbon number separation on the C_{18} phase of prim., sec. and tert. anilines, respectively, isolated from the run of the total mixture on the nitrile phase (Fig. 3). Stationary phase, Nucleosil C_{18} ; mobile phase, methanol-aqueous buffer (80:20, v/v, pH = 7.0); $45 \text{ ml} \cdot \text{h}^{-1}$. P = prim. anilines; S = sec. anilines; T = tert. anilines. The numbers refer to Table VII

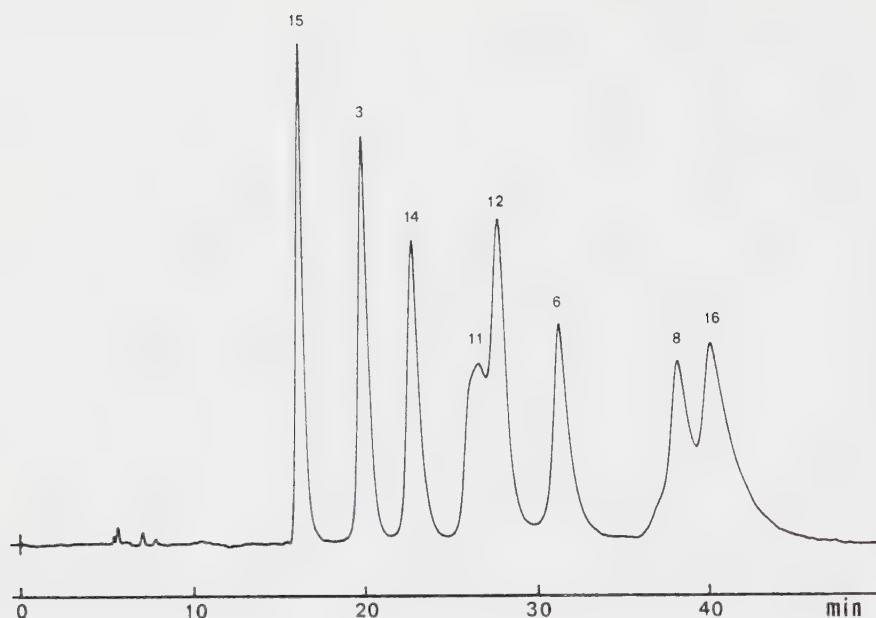


Fig. 6. Separation on the nitrile phase of prim. anilines, present in the peak with carbon number two, isolated from the carbon number run of prim. anilines (Fig. 5). Stationary phase, Nucleosil CN; mobile phase, 0.2% (v/v) 2-propanol in isooctane; $45 \text{ ml} \cdot \text{h}^{-1}$. The numbers refer to Table VII

The tertiary fraction isolated from the nitrile phase can contain some sec. anilines which, however, can be separated from the tertiary ones by treatment with acetic anhydride. The acetylation mixture is separated on a short nitrile phase-containing column. The tert. anilines are rapidly eluted with isooctane, while the acetylated sec. anilines require a stronger solvent, *viz.* ethanol, for rapid elution. After hydrolysis of the acetylated sec. anilines, the free sec. anilines can be chromatographed on the nitrile column, as can the tert. anilines from the mixture.

BROMINATED ALKYLANILINES (IV, V)

These papers describe the application of HPLC to the analysis of products formed at coulometric bromination of alkyilanilines and include a detailed study of retention behaviour of brominated ani-

lines. The combination of HPLC and coulometric bromination by the method described in (I) offers a unique possibility for the study of retention characteristics of brominated anilines since a large number of *ortho*- and *para*-brominated anilines can be readily synthesized. The main bromination reaction involves exchange of hydrogen for bromine in free *ortho*- and *para*-positions and the coulometric methods generally yield predictable, unambiguous products for the anilines investigated up to the end-point.

After the end-point, over-bromination of prim. anilines leads to oxidation products, particularly for the *ortho*-alkyl-substituted anilines where *p*-amino-substituted diphenylamines among other products are formed. The sec. and tert. anilines are generally not fullbrominated at the end-point. On over-bromination, these compounds are substituted with bromine in the remaining free *ortho*- and *para*-positions and, on further bromination, loss of N-alkyl groups occurs with the formation of prim. and sec. anilines.

The studies in paper (IV) concern anilines without ring substituents or with alkyl groups in the *ortho*- and *para*-positions, while anilines with alkyl groups in the *meta*-position are treated in paper (V). Both straight- and reversed-phase LC have been used for these studies. For all anilines investigated, it turned out that an increase in retention generally took place on the introduction of bromine into the aniline nucleus, but for the formation of *o*-bromoanilines and most of the *para*-brominated tert. anilines in straight-phase LC, where retention decreased (see Tables VIII, IX, X and XI).

There exists for both systems a semi-linear relationship between k' values of original anilines on one hand, and k' values of the corresponding mono-, di- and tribrominated derivatives, respectively, on the other hand.

On the straight-phase LC system, the decrease in retention following *ortho*-bromination is considered to be due to a decrease in base strength and to increased steric hindrance to interaction between the amino group and the bonded phase nitrile group. The general increase in retention on *para*-bromination of prim. and sec. anilines is thought to be due to a decreased solubility of the *p*-bromoaniline in the mobile phase.

Table VIII. Capacity factors, k' , and separation factors, α , for *ortho*- and *para*-alkyl-substituted anilines and their bromination products in the reversed-phase LC system

Column: Nucleosil C₁₈. Eluent: methanol-aqueous buffer (80:20 and 70:30*, v/v, pH = 7.0)

No.	Aniline (Substituent)	k'		α							
		Non- brominated	Monobrominated Ortho	Para	Dibrominated Di-ortho Ortho-para	Tri- brominated	Mono-/non- Ortho	Di-/mono-ortho- Para	Di-/mono-ortho- Di-ortho	Di-/mono-ortho- Ortho-para	Tri-/
1	None*	0.55	1.33	1.16	3.33	8.87	2.42	2.11	2.50	2.87	2.66
1	None	0.41				3.48					
2	2-Methyl*	0.85	2.20	1.82	5.68		2.59	2.14	2.58	3.12	
2	2-Methyl	0.55	1.10	0.96	2.35		2.00	1.75	2.14	2.45	
3	2-Ethyl*	1.26	3.14	2.54	7.87		2.49	2.02	2.51	3.10	
4	2-Isopropyl*	1.69	4.20	3.33	10.2		2.49	1.97	2.43	3.06	
5	4-Methyl*	0.86	2.01		5.36		2.34		2.67		
6	4-Ethyl*	1.26	3.04		8.25		2.41		2.71		
7	4-Isopropyl*	1.80	4.08		10.8		2.27		2.65		
8	4-n-Butyl*	3.29	7.84		21.5		2.38		2.74		
9	2,4-Dimethyl*	1.25	3.36				2.69				
10	2-Methyl-4-butyl*	5.02	12.7				2.53				
11	2,6-Dimethyl*	1.31		2.89				2.21			
11	2,6-Dimethyl	0.78		1.39				1.78			
12	N-Methyl	0.68		1.25	3.04	5.14		1.84		2.43	1.69
13	N-Ethyl	0.87		1.57	4.39	7.18		1.80		2.80	1.64
14	N-Propyl	1.23		2.26	6.46	11.3		1.84		2.86	1.75
15	N-n-Butyl	1.69		2.79	8.87	15.3		1.65		3.18	1.72
16	N-Phenyl	1.52	3.20	2.79	4.89 ⁺	4.71 [§]	2.11	1.84			
17	N-Benzyl	1.50		2.61	6.55	12.3		1.74		2.51	1.88
18	N-Ethyl-2-methyl	0.97		1.83	3.80			1.89		2.08	
19	N-Ethyl-2-methyl	1.34		2.48	5.30			1.85		2.14	
20	N-Ethyl-2-ethyl	1.86		3.23	6.90			1.74		2.14	
21	N-Methyl-4-methyl	0.90	2.17		2.95		2.41		1.36		
22	N-Ethyl-4-methyl	1.15	3.26		4.20		2.83		1.29		
23	N,N-Dimethyl	1.35		2.45	3.64	5.02		1.81		1.49	1.38
24	N,N-Diethyl	2.32		4.02	7.06			1.73		1.76	
25	N,N-Diphenyl	7.40		13.0	21.4 [§]	31.9 ⁺		1.76		1.65	1.49
26	N,N-Dimethyl- -2-methyl	1.76		3.20	8.91			1.82		2.78	
27	N,N-Diethyl- -2-methyl	3.45		6.40	16.2			1.86		2.53	
28	N,N-Diethyl- -2-ethyl	5.21		8.94	21.8			1.72		2.44	
29	N,N-Dimethyl- -4-methyl	1.80	2.32		7.37		1.29		3.18		
30	N,N-Diethyl- -4-methyl	2.95	4.61		13.5		1.56		2.93		
31	N,N-Dimethyl- -2,6-dimethyl	4.20		7.84				1.87			

⁺2,4'-dibrominated

[§]4,4'-dibrominated

^{*}2,4,4'-tribrominated; 2,4,2',4'-tetrabrominated; $k' = 2.03$

⁺4,4',4''-tribrominated

Table IX. Capacity factors, k' , and separation factors, α , for *ortho*- and *para*-alkyl-substituted anilines and their bromination products in the straight-phase LC system

Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane

No.	Aniline (Substituent)	k'	α											
			Non- brominated	Monobrominated Ortho	Para	Dibrominated Di-ortho	Ortho-para	Tri- brominated	Mono-/non- Ortho	Para	Di-/mono-ortho- Di-ortho	Ortho-para	Di-/mono-para- Ortho-para	Tri-/di- Ortho-para
1	None	7.53		2.65	10.7		3.96	0.87	0.35	1.42		1.49	0.37	0.22
2	2-Methyl	4.84		1.41	6.74		2.06		0.29	1.39		1.46	0.31	
3	2-Ethyl	3.65		1.13	5.20		1.57		0.31	1.42		1.39	0.30	
4	2-Isopropyl	3.14		0.93	4.45		1.28		0.30	1.42		1.38	0.29	
5	4-Methyl	9.62		2.50		0.67			0.26		0.27			
6	4-Ethyl	8.72		2.26		0.60			0.26		0.27			
7	4-Isopropyl	8.87		2.16		0.57			0.24		0.26			
8	4-n-Butyl	8.72		2.11		0.54			0.24		0.26			
9	2,4-Dimethyl	6.04		1.36					0.23					
10	2-Methyl-4-butyl	5.07		1.20					0.24					
11	2,6-Dimethyl	2.83			3.91					1.38				
12	N-Methyl	2.32			3.19		0.87	0.42		1.38			0.27	0.48
13	N-Ethyl	1.49			1.98		0.51	0.29		1.33			0.26	0.57
14	N-Propyl	1.06			1.50		0.41	0.25		1.42			0.27	0.61
15	N-n-Butyl	0.90			1.27		0.37	0.23		1.41			0.29	0.62
16	N-Phenyl	2.50	0.61		6.89	0.87 ⁺	4.01 [§]	1.04 [*]	0.24	2.76				
17	N-Benzyl	1.65			2.62		1.43	0.43		1.59			0.55	0.30
18	N-Methyl-2-methyl	1.43			2.01		0.58			1.41			0.29	
19	N-Ethyl-2-methyl	0.82			1.11		0.39			1.35			0.35	
20	N-Ethyl-2-ethyl	0.67			0.90		0.32			1.34			0.36	
21	N-Methyl-4-methyl	2.73	0.61			0.53			0.22		0.87			
22	N-Ethyl-4-methyl	1.72	0.39			0.38			0.23		0.97			
23	N,N-Dimethyl	0.65			0.73		0.44	0.14		1.12			0.60	0.32
24	N,N-Diethyl	0.50			0.48		0.24			0.96			0.50	
25	N,N-Diphenyl	0.39			0.52		0.46 [§]	0.41 [‡]		1.33			0.88	0.89
26	N,N-Dimethyl- -2-methyl	0.45			0.42		0.15			0.93			0.36	
27	N,N-Diethyl- -2-methyl	0.29			0.22		0.13			0.76			0.59	
28	N,N-Diethyl- -2-ethyl	0.21			0.18		0.11			0.86			0.61	
29	N,N-Dimethyl- -4-methyl	0.79	0.47			0.18			0.59		0.38			
30	N,N-Diethyl- -4-methyl	0.90	0.24			0.15			0.27		0.63			
31	N,N-Dimethyl- -2,6-dimethyl	0.14			0.18					1.29				

⁺2,4'-dibrominated

[§]4,4'-dibrominated

^{*}2,4,4'-tribrominated; 2,4,2',4'-tetrabrominated; $k' = 0.44$

[‡]4,4',4''-tribrominated

Table X. Capacity factors, k' , and separation factors, α , for *meta*-alkyl-substituted anilines and their bromination products in the reversed-phase LC system

Column: Nucleosil C₁₈. Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0)

No.	Aniline (Substituent)	k'				α					
		Non-brominated	Monobrominated Ortho	Para	Dibrominated 2,6- 2,4- 4,6-	Tri-brominated	Mono-/non-	Di-/mono-	para-	Tri-/di-	
1	3-Methyl	0.51		0.88	2.17 2.17 5.14		1.73	2.47	2.47	2.37	2.37
2	3-Ethyl	0.67	1.31	1.12	2.57 2.73 6.24		1.96	1.67	2.29	2.44	2.43 2.29
3	2,3-Dimethyl	0.74		1.25	3.23		1.69		2.58		
4	2,5-Dimethyl	0.75		1.29	3.26		1.72		2.53		
5	3,4-Dimethyl	0.69	1.37		3.14		1.99				
6	N-Methyl-3-methyl	0.89		1.66	4.33 4.24 7.18		1.87	2.61	2.55	1.66	1.69
7	N-Ethyl-3-methyl	1.14		2.11	6.55 6.30 10.3		1.85	3.10	2.99	1.57	1.63
8	N,N-Dimethyl-3-methyl	1.82		3.42	5.18 17.7		1.88		1.51		3.42
9	N,N-Diethyl-3-methyl	3.08		5.83	10.1 31.9		1.89		1.73		3.16

Table XI. Capacity factors, k' , and separation factors, α , for *meta*-alkyl-substituted anilines and their bromination products in the straight-phase LC system

Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane

No.	Aniline (Substituent)	k'				α					
		Non-brominated	Monobrominated Ortho	Para	Dibrominated 2,6- 2,4- 4,6-	Tri-brominated	Mono-/non-	Di-/mono-	para-	Tri-/di-	
1	3-Methyl	8.07		9.62	3.06 3.06 0.68		1.19	0.32	0.32	0.22	0.22
2	3-Ethyl	7.02	1.93	8.31	2.93 2.57 0.60		0.27	1.18	0.35	0.31	0.20 0.23
3	2,3-Dimethyl	5.48		7.15	1.88		1.30		0.26		
4	2,5-Dimethyl	4.45		5.50	1.57		1.24		0.29		
5	3,4-Dimethyl	9.51	2.24		0.60		0.24				
6	N-Methyl-3-methyl	2.16		2.62	0.66 0.73 0.37		1.21	0.25	0.28	0.56	0.51
7	N-Ethyl-3-methyl	1.40		1.65	0.42 0.45 0.28		1.18	0.25	0.27	0.67	0.62
8	N,N-Dimethyl-3-methyl	0.68		0.42	0.37 0.11		0.62		0.88		0.30
9	N,N-Diethyl-3-methyl	0.54		0.42	0.21 0.08		0.78		0.50		0.38

The decrease in retention found for the main part of the *para*-brominated tert. anilines is most likely a result of the interplay between the change in base strength and the change in solubility, on bromination. Also the different behaviour of certain sec. anilines, on introduction of a second and third bromine atom, compared to corresponding prim. and tert. anilines may well be caused by solubility differences.

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A GENERAL METHOD FOR COULOMETRIC TITRATION OF ALKYLANILINES WITH BROMINE

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Summary—A general method is described for the coulometric titration of alkyranilines with anodically generated bromine. The reaction is carried out in water-acetic acid medium and the reactivity is governed by varying the water content and the concentration of bromide ion and by the addition of pyridine. In this way most types of alkyranilines can be titrated quantitatively. Unlike the case for alkyphenols, the bromine consumption is not always higher in the pyridine-containing media than in the pyridine-free. The bromine consumption is also more dependent on the bromide content of the medium than it is for alkyphenols. The mean relative error, for the best medium for each compound, is $\pm 0.9\%$ for primary alkyranilines, $\pm 0.9\%$ for secondary and $\pm 1.2\%$ for tertiary.

Recently a general method for the coulometric titration of alkyphenols with anodically generated bromine was described in this journal.¹ The present communication deals with a related problem, the coulometric bromination of alkyranilines. Bromination has historically been an important method for the quantitative determination of anilines, especially aniline itself and methylanilines. Non-coulometric bromination methods have utilized direct titration with bromine or bromide-bromate solutions and the endpoint has been detected visually, with or without an internal indicator.^{2,3} Starch-iodide paper as an external indicator has also often been employed⁴ as have instrumental indication methods, *e.g.*, potentiometric⁵ and photometric⁶ methods. The most common approach, however, is to use a back-titration where titrant is added in excess and the excess is then determined iodometrically.⁷ The reason for the frequent application of the back-titration method is the sluggish reaction of many anilines with bromine under the conditions generally used.

The variety of methods employed demonstrates the difficulty of developing a general bromination method for alkyranilines. An example of this is given by Day and Taggart⁸ who tried in vain to develop universally applicable bromination methods for phenols and aromatic amines. Of a number of alkyranilines investigated, only aniline and *m*-toluidine could be determined with sufficient accuracy, although direct as well as indirect methods were utilized. In a more recent investigation Matrka *et al.*⁹ studied the quantitative bromination of several *N*-alkylanilines in 1M hydrochloric acid. The direct bromination method was too slow to be useful and only *N*-methylaniline could be determined by the back-titration method. Oxidative side-reactions also took place whereby alkyl groups were split off. The reason for the problems encountered is thought to lie mainly in the choice of titration medium. Although aqueous media are most common,

brominations of alkyranilines have also been performed in glacial acetic acid¹⁰ and in propylene carbonate.¹¹

Examples of coulometric bromination methods applied to alkyranilines are scarce. Buck and Swift¹² seem to have been the first to utilize a coulometric bromometric titration method for the determination of aniline. Bromine was generated in excess, and back-titrated with electrolytically generated copper(I). The titration medium was an acidified aqueous solution. The back-titration mode was necessitated by the slow reaction between aniline and bromine under the conditions used. In a more extensive coulometric investigation Delgado¹³ titrated aniline and *o*- and *p*-toluidine. The reaction was governed by regulation of the pH. Thus, while aniline could be quantitatively brominated in the pH range 1–5, the toluidines had to be titrated at pH 1. *N,N*-Dimethylaniline could not be titrated because the reaction was too slow. The titration was followed biamperometrically with two platinum electrodes and the indicator currents were plotted manually against time.

The aim of the present investigation was to develop a general coulometric method usable for the quantitative determination of all kinds of alkyranilines. In our previous communication¹ an extensive study was made of various factors influencing the quantitative bromination reaction of phenols, *i.e.*, composition of titration medium, amount of substance titrated and magnitude of generating current and polarizing resistance. It was found that the conclusions drawn from this investigation were also valid for the quantitative coulometric bromination of alkyranilines. Accordingly, aqueous acetic acid was used as titration medium. The reactivity-promoting properties of the media were regulated by varying the proportions of acetic acid and water and the bromide concentration. To some media pyridine was added in order to accelerate the bromination reaction (see Table 1).

Table 1. Composition of media used for bromination of anilines

Medium	Acetic acid, % v/v	Water, % v/v	Pyridine, % v/v	[Br ⁻], M
I-1	55	35	10	0.1
I-2	55	35	10	0.4
I-3	55	35	10	1.2
II-3	55	40	5	1.2
III-1	60	40	—	0.1

EXPERIMENTAL

Apparatus

The apparatus has already been described¹ and was used without change. The generating current was 3 mA, the polarizing resistance 100 kΩ and the chart-speed 30 mm/min.

Reagents

Anilines. These were of the best grade commercially available. Some of them were further purified by distillation or recrystallization. *N,N*-2,6-Tetramethylaniline and *N,N*-2-triethylaniline were prepared in the laboratory.¹⁴

Acetic acid. Merck, 99–100%.

Sodium bromide. Sodium bromide, 99%, BDH.

Pyridine. Mallinckrodt analytical grade.

Titration procedure

The details have already been published,¹ the only change being use of methanol instead of acetic acid for dissolving the sample. The reason for this is the risk of partial acetylation of primary and secondary anilines with the formation of unreactive *N*-acetylanilines. An amount of amine was taken each time which would consume between 10 and 20 μeq of bromine (*i.e.*, required 5–10 min generation at 3 mA).

RESULTS AND DISCUSSION

Thirty-nine anilines were titrated by the coulometric bromination method. Of these 17 were primary

anilines (Table 2), 12 secondary anilines (Table 3) and 10 tertiary anilines (Table 4). The results of the brominations have been summarized in Table 5 and are the means of at least two titrations.

Primary anilines

All the primary anilines investigated could be titrated in the pyridine-free medium III-1, containing 60% v/v acetic acid and 0.1M with respect to bromide (see Table 1). The reaction between aniline and bromine is somewhat sluggish in this medium, giving low results. On that account the pyridine-containing media I-1 or II-3 are recommended. As seen from Table 2, all free *ortho* and *para* positions are substituted by bromine whether the medium contains pyridine or not. It is noteworthy that pyridine is not necessary for full bromination. In the previous investigation of phenols it was generally found that monobromination took place in pyridine-free media, while a pyridine-containing medium was required for full bromination.

In this case it was even found that less bromine was consumed by aniline in the pyridine-containing medium II-3 (2 moles of Br/mole) than in the pyridine-free medium III-1 (3 moles of Br/mole) and the titration could be carried out with a precision better

Table 2. Results of bromination of primary alkylanilines

Aniline	Titn. medium	Number of H substituted	Mean error, %	Titn. medium	Number of H substituted	Mean error, %
C ₆ H ₅ NH ₂	III-1	3	-4.5	I-1	3	-1.6
	—	—	—	II-3	2	-0.8
C ₆ H ₅ NH ₂ , HCl	III-1	3	-4.0	I-1	3	-2.5
2-Me	III-1	2	+0.4			
2-Et	III-1	2	-1.0			
2-isoPr	III-1	2	-0.8			
3-Me	III-1	3	+0.1	I-1	3	-0.5
3-Et	III-1	3	-0.1			
3-Et, HCl	III-1	3	-0.9			
4-Me	III-1	2	-0.3	I-1	2	+0.4
4-Et	III-1	2	+0.8			
4-Et, HCl	III-1	2	-0.9			
4-isoPr	III-1	2	-0.8			
4-Bu	III-1	2	-0.2	I-1	2	-0.2
2,3-diMe	III-1	2	-0.4			
2,4-diMe	III-1	1	+1.1			
2-Me-4-Bu	III-1	1	-2.5	I-3	1	-1.6
2,5-diMe	III-1	2	-0.7			
2,6-diMe	III-1	1	+0.8			
3,4-diMe	III-1	2	+0.5			
2,4,6-triMe	III-1	0	—	I-1	1	+3.4

Table 3. Results of bromination of secondary alkylanilines

Aniline	Titn. medium	Number of H substituted	Mean error, %	Titn. medium	Number of H substituted	Mean error, %
<i>N</i> -Me	III-1	2	-0.6	I-2	2	-0.6
<i>N</i> -Et	III-1	2	-1.4	I-2	2	-0.8
<i>N</i> -Pr	III-1	2	-1.3	I-3	2	-0.8
<i>N</i> -Bu	III-1	2	-1.3	I-2	2	-1.0
<i>N</i> -Bz	III-1	2	-2.8	I-1	2	-1.9
<i>N</i> -Ph	III-1	4	-3.7	I-1	4	-3.0
<i>N</i> -Me-2-Me	III-1	2	*	I-1	2	-0.6
<i>N</i> -Et-2-Me	III-1	1	+2.1	I-1	2	+0.1
<i>N</i> -Me-4-Me	III-1	2	*	I-1	2	+1.0
<i>N</i> -Et-4-Me	III-1	0	—	I-1	2	-0.2
<i>N</i> -Et-3-Me	III-1	2	-0.6	I-1	3	-1.4
<i>N</i> -Me-3-Me	III-1	2	+0.8	I-1	3	-2.8
				I-3	2	-1.3

* Sloping titration curve, difficult to evaluate.

than 1%. We believe this fact to be due to the higher bromide content in the former case (1.2*M* against 0.1*M*) which for anilines has a greater influence on the reactivity than the presence or absence of pyridine.

2,4,6-Trimethylaniline does not react with bromine in the pyridine-free medium III-1, but in the pyridine-containing medium I-1 it consumes one mole of bromine per mole of aniline. The reaction of this compound with bromine, in spite of the fact that it has no free *ortho* and *para* positions, should be compared with the corresponding reaction between 2,4,6-trimethylphenol and bromine. In the latter case a *para*-quinoid bromocyclohexadienone is formed and it is believed that an analogous compound results from the reaction between 2,4,6-trimethylaniline and bromine. Although in most cases results of similar accuracy were obtained in media with and without pyridine, it was often observed that the titration curve in the pyridine-containing media was steeper after the end-point, which facilitated the detection of the end-point (cf. Fig. 1 in ref. 1).

The reaction between some primary anilines and bromine in medium IV,¹ containing 10% v/v water but no pyridine, was also studied. It appeared that in this medium aniline, 2-methylaniline and 3-methylaniline were monobrominated while 4-methylaniline did not react, indicating that the bromine entered the *para* position. However, the results are not communicated in Table 2 as they were only approximate. This difference in reactivity could be used to distinguish between 2- and 3-alkylanilines on the one hand and 4-alkylanilines on the other.

Table 2 also includes some titration results for hydrochlorides of primary anilines. As might be expected, they can be titrated just as well as the free amines in acetic acid-water media. This is of interest as anilines are often isolated and purified as their hydrochlorides.

Secondary anilines

Secondary anilines, like the primary, can be titrated in medium III-1 (with some exceptions) or in one of the media of group I (see Table 3). However, the behaviour of secondary anilines is different from that of the primary ones. Thus, in secondary anilines without nuclear substituents bromine generally enters only two of the three vacant *ortho* and *para* positions, irrespective of whether the medium contains pyridine (I) or not (III-1). For secondary anilines with nuclear alkyl groups in the *ortho* or *para* positions a further decreased reactivity is observed in medium III-1. This is sometimes shown by a sloping titration curve, which makes it difficult to evaluate the end-point accurately or, as for *N*-ethyl-4-methylaniline, by absence of reaction. Secondary anilines with a methyl group in the *meta* position are as reactive in medium III-1 as secondary anilines without nuclear alkyl groups, bromine entering two of the three vacant *ortho* and *para* positions.

In the pyridine-containing medium I-1 the reactivity of the nuclear-substituted secondary anilines is greater than in medium III-1. Thus all compounds gave some reaction, and all hydrogen atoms in free *ortho* and *para* positions were exchanged for bromine as compared to one hydrogen atom less in medium III-1 for the anilines which did react in this medium. Another example of the influence of the bromide concentration in the medium is given by the reaction of *N*-methyl-3-methylaniline for which the number of entering bromine atoms is lowered from three to two when the bromide concentration is increased from 0.1*M* (I-1) to 1.2*M* (I-3).

Tertiary anilines

The decreased reactivity towards bromine previously observed for secondary anilines is further accentuated for tertiary anilines (see Table 4). Thus

Table 4. Results of bromination of tertiary alkylanilines

Aniline	Titn. medium	Number of H substituted	Mean error, %	Titn. medium	Number of H substituted	Mean error, %
<i>N,N</i> -diMe	III-1	1	+1.0	I-1	2	±0.0
<i>N,N</i> -diEt	III-1	1	-1.3	I-3	1	+2.3
<i>N,N</i> -diPh	III-1	3	-0.6	I-1	3	-0.2
<i>N,N</i> -diMe-2-Me	III-1	0	—	I-2	1	+2.8
<i>N,N</i> -diEt-2-Me	III-1	0	—	I-1	0	—
<i>N,N</i> -diEt-2-Et	III-1	0	—	I-1	0	—
<i>N,N</i> -diMe-3-Me	III-1	1	-0.7	I-1	2	-0.6
<i>N,N</i> -diEt-3-Me	III-1	1	-1.2	I-1	2	-3.7
<i>N,N</i> -diMe-4-Me	III-1	0	—	I-1	1	+3.6
<i>N,N</i> -diEt-4-Me	III-1	0	—	I-1	1	+0.1

in the pyridine-free medium III-1 only compounds without nuclear substituents or with a methyl group in the *meta* position react. Only one bromine atom enters each benzene ring, probably in the *para* position.

In the pyridine-containing media I, all the compounds tested except two, namely *N,N*-diethyl-2-methylaniline and *N,N*-diethyl-2-ethyl-aniline gave some reaction. The number of hydrogen atoms substituted varies with the structure. In tertiary anilines without nuclear substituents, bromine enters one or two of the three vacant *ortho* or *para* positions, depending on the size of the groups at the nitrogen atom. Thus, in *N,N*-dimethylaniline two hydrogen atoms are exchanged and the titration can be performed in medium I-1. Attempts to titrate *N,N*-diethylaniline in the same medium showed that approximately two moles of bromine were consumed per mole of determinant, but the values were too low to be useful for quantitative determination. However, a change to medium I-3 where one mole of bromine was consumed per mole, gave a quantitative result. We have here another example of the marked influence of the bromide concentration in the medium on the bromine consumption. As can be seen from Table 4, the pyridine-free medium III-1, in which the reacting ratio is likewise 1:1, is the best choice for the determination of this compound. The third tertiary aniline without nuclear substituents in Table 4, triphenylamine, consumes one molecule of bromine per benzene ring in the most bromination-promoting medium I-1 just as in medium III-1.

Of the nuclear-substituted tertiary anilines, those with substituents at the *ortho* and *para* positions consume one mole of bromine per mole or do not react at all in media of group I. The unreactive compounds, *N,N*-diethyl-2-methylaniline and *N,N*-diethyl-2-ethyl-aniline are the only anilines in this investigation which did not react in any of the media investigated. Obviously anilines in which the steric interaction between the substituents at the nitrogen atom and an *ortho*-situated group is great enough must be expected to be unreactive towards bromine. The two nuclear-substituted compounds with methyl groups in

the *meta* position consume two moles of bromine per mole in medium I-1 against one in medium III-1.

In addition to the tertiary anilines listed in Table 4, *N,N*-dimethyl-2,6-dimethylaniline was also titrated with bromine. This compound did not react in the pyridine-free medium III-1, but consumed between three and four moles of bromine per mole in the pyridine-containing medium I-1. However, the latter reaction, which we believe to be due to oxidation, is not useful for quantitative determination.

The secondary and tertiary anilines are less reactive than the primary anilines because of the steric interaction between substituents on the nitrogen atom and *ortho*-situated groups existent in these compounds or in their bromination products. The nuclear substituent can be an alkyl group or a bromine atom. It is known that this steric hindrance interferes with the alignment between the nitrogen atom and the plane of the benzene ring, thereby decreasing the activation which arises from resonance of the *ortho* and *para* positions.¹⁵

In Table 5 the results of the bromination of aniline and alkylanilines have been summarized. The decreased reactivity towards bromine in the series primary-secondary-tertiary anilines is clearly demonstrated. It is also seen that pyridine exerts a bromination-promoting effect only for secondary anilines with nuclear substituents and for tertiary anilines. Likewise it is evident that among nuclear-substituted secondary and tertiary anilines the *meta*-substituted are generally most reactive.

Differentiation between anilines by acetylation and bromine titration

When a primary or secondary aniline is acetylated, it loses its ability to react with bromine in the coulometric titration method. This fact can be utilized for distinguishing these types of anilines from tertiary ones. Thus an aniline which can react with bromine before acetylation but not after heating for a couple of minutes with an excess of acetic anhydride, is either primary or secondary. Because of the possibility of acetylation the use of aqueous acetic acid as titration medium can be questioned. However, we have not

Table 5. Media for quantitative bromination of alkyilanilines

Type of aniline	Medium	Number of available hydrogen atoms exchanged for bromine
Primary		
With and without substituents	III-1 or I II-3*	All 2 of 3
Secondary		
Without nuclear substituents	III-1 or I	2 of 3
With nuclear substituents in 3-position	III-1 I-1	2 of 3 All (3)
in 2- or 4-position	III-1 I-1	Not recommended† All (2)
Tertiary		
Without nuclear substituents	III-1 I	1 of 3 2 or 1 of 3
With nuclear substituents in 3-position	III-1 I-1	1 of 3 2 of 3
in 2- or 4-position	III-1 I	None 1 of 2, or none

* Aniline.

† See Table 3.

noted any adverse effects of this titration medium on the bromination reaction of primary and secondary anilines and consequently conclude that no acetylation takes place under the conditions used (see also "Experimental").

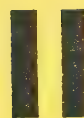
CONCLUSIONS

It is concluded that nearly all kinds of alkyilanilines can be titrated quantitatively with coulometrically generated bromine in water-acetic acid media, with or without added pyridine. The standard titration media are the pyridine-free medium III-1 and the pyridine-containing media of group I. The former is well suited for the determination of primary anilines except aniline while secondary and tertiary anilines are better determined in the pyridine-containing media. Not all of the anilines investigated can be determined. Tertiary anilines with alkyl groups in one or both of the *ortho* positions may give no reaction, or may react to give irreproducible results, as exemplified by some compounds in Table 4.

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COULOMETRIC TITRATION OF ANILINES, MAINLY CONTAINING DEACTIVATING FUNCTIONAL GROUPS, WITH BROMINE

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Summary—A general method is described for the coulometric titration of various substituted anilines with anodically generated bromine. The reaction is carried out in water–acetic acid medium and the reactivity is governed by varying the water content and the bromide concentration, and by the addition of pyridine. In this way the majority of the substituted anilines tested can be titrated quantitatively. There is no great difference in the reactivity-promoting properties of pyridine-free and pyridine-containing media, but the latter generally give the better results and are therefore to be preferred. The mean relative error, for the best medium for each compound, is $\pm 0.5\%$ for the haloanilines and $\pm 1.0\%$ for anilines with oxygenated functional groups.

In a previous paper¹ a general method was described for the coulometric titration of alkyranilines with anodically generated bromine. This paper deals with the coulometric bromination of anilines, mainly those containing deactivating groups. Non-coulometric bromination methods have previously been applied to several deactivated anilines with varying success, while coulometric bromination methods have been used only very occasionally. Kalinowski and Zwierzchowski^{2,3} seem to have been the first to utilize coulometric bromometric titration for the determination of deactivated anilines. They titrated *p*-aminobenzoic acid and novocaine, an ester of *p*-aminobenzoic acid, in aqueous sulphuric acid. The titration was followed biamperometrically with two platinum electrodes and either a known excess of bromine was generated, or the substance was titrated directly to the end-point. Delgado⁴ titrated *o*- and *p*-aminobenzoic acid and sulphanilic acid, likewise in aqueous solution, and the reaction was governed by regulation of the pH. The titration was followed biamperometrically with two platinum electrodes and the indicator currents were plotted manually against time.

In this investigation a thorough study has been made of the application of the coulometric bromometric titration method, originally developed for phenols,^{5,6} to the determination of anilines containing various functional groups, mainly deactivating. The following groups are included: halogen, methoxy, formyl, acetyl, carboxyl, ester, carboxymethyl (CH_2COOH), nitro and sulphonate. Aqueous acetic acid was used as titration medium for the majority of the compounds investigated and the reactivity-promoting properties of the media were regulated by varying the content of acetic acid and water and the concentration of bromide ion. To some media pyridine was added in order to promote the bromination reaction (see Table 1).

EXPERIMENTAL

Apparatus

The apparatus has been described previously⁵ and was used without change. The generating current was 3 mA, the polarizing resistance 100 k Ω and the chart-speed 30 mm/min.

Reagents

The anilines used were of the best grade commercially available. Some of them were further purified by distillation or recrystallization. The other reagents were as described before.¹

Titration procedure

For details of the titration procedure, see the earlier work,⁵ the only change being use of methanol instead of acetic acid for dissolving the sample, because of the risk of acetylation of primary and secondary anilines with the formation of non-reactive acetanilides.¹ In the case of aminosulphonic acids water is used as the solvent.

An amount of amine is weighed out which is calculated to consume about 20 μeq of bromine for full bromination. Generally the break in the titration curve corresponding to full bromination is taken as the end-point. In most instances the results are not much dependent on the amount of substance titrated, provided that the bromine consumption lies in the range 10–20 μeq .

In cases where the titration curve has two breaks it is sometimes necessary to use the first for evaluation of the end-point. The break used is given in the appropriate column in the tables, in parentheses. The number of hydrogen atoms substituted given in the tables always corresponds to the break utilized for end-point evaluation, irrespective of whether the first or second break is used.

There are two main reasons for failure of the bromometric titration method, and these are evident from the appearance of the titration curve. Thus, the break in the titration curve, although reproducible and corresponding to the consumption of a whole-number reaction ratio, may be too small for accurate evaluation of the end-point. Another cause of failure is that the break, although steep enough, is not reproducible or gives erroneous results. The results given in the tables are the average of at least two titrations. As seen from the column giving the number of hydrogen atoms substituted, some amines are only partly

Table 1. Composition of media used for bromination of anilines

Medium	Acetic acid, % v/v	Water, % v/v	Pyridine, % v/v	[Br ⁻], M
I-1	55	35	10	0.1
I-2	55	35	10	0.4
I-3	55	35	10	1.2
III-1	60	40	—	0.1

substituted by bromine at the free *ortho* and *para* positions by the time the end-point is reached, but more bromine is often consumed after this, since the titration curve does not immediately return to the baseline after the end-point (see Tables 2 and 3).

RESULTS AND DISCUSSION

About fifty substituted anilines were titrated in medium III-1 and also in one or several of the media of group I (see Table 1). Nearly all the anilines were primary, and only two tertiary anilines were included. In a previous study on the coulometric bromometric titration of phenols containing deactivating functional groups⁶ it was found that these compounds required pyridine-containing media, *i.e.* from groups I or II, for quantitative bromination. For the corresponding anilines this is not the case. They can be titrated in

pyridine-free as well as pyridine-containing media, but the best results were generally obtained in the latter, which accordingly are to be preferred.

Another difference between the behaviour of phenols and anilines containing deactivating groups was that while the strongly deactivated phenols such as trichloro- and dinitrophenols could be quantitatively brominated, this was not the case for the corresponding anilines. The latter compounds either did not consume bromine at all or gave sloping titration curves from which the end-point could not be properly evaluated. The marked dependence of the reactivity of anilines on the bromide concentration in the medium, which was previously observed for alkylanilines,¹ was found also to exist for the types of anilines studied in this work.

Haloanilines

Most haloanilines can be titrated with fair accuracy in the pyridine-containing media I-1 and/or I-3 (see Table 2). For comparison, a titration was also performed in the pyridine-free medium III-1. Although results of comparable accuracy are reported in several cases for media of groups I and III in Table 2, medium III-1 tended to give sloping titration curves which were difficult to evaluate accurately.

As can be seen from Table 2, *ortho*- and *para*-sub-

Table 2*. Results of bromination of haloanilines

Aniline	Titn. medium	Number of H substituted	Number of breaks in the titration curve	Mean relative error, %	Titn. medium	Number of H substituted	Number of breaks in the titration curve	Mean relative error, %
2-Fluoro	I-1	2	1	-0.8	III-1	2	1	-2.6
3-Fluoro	I-3	2	1	-0.5	III-1	2	1	+2.3
4-Fluoro	I-1	2	1	-0.2	III-1	1-2	1	†
2,4-Difluoro	I-1	1	1	-0.6	III-1	~1	1	†
2,5-Difluoro	I-3	1	1	+0.4	III-1	1	1§	+1.7
2-Chloro	I-1	2	2(2)	+0.3	III-1	2	2(2)	-1.5
2-Chloro-6-methyl	I-1	1	1	+0.2	III-1	1	1	+0.3
3-Chloro	I-3	2	1	-0.3	III-1	2	2(1)§	+0.3
3-Chloro-2-methyl	I-1	2	1	-0.6	III-1	2	1	-0.5
3-Chloro-4-methyl	I-1	2	1	-0.7	III-1	2	1	-1.3
5-Chloro-2-methyl	I-1	2	1	+0.4	III-1	2	1	-0.3
4-Chloro	I-1	2	2(2)	-0.8	III-1	~2	1	†
2,3-Dichloro	I-3	1	1§	+0.4	III-1	~1	2(1)§	†
2,4-Dichloro	I-1	1	1	+0.1	III-1	~1	1	†
2,5-Dichloro	I-3	1	1	-0.7	III-1	1	2(1)§	+0.4
2,6-Dichloro	I-1	1	1	+1.0	III-1	1	1	-0.7
3,4-Dichloro	I-3	1	1	+1.0	III-1	~1	2(1)§	†
3,5-Dichloro	I-1	2	1§	+0.6	III-1	2	1	+0.1
2,3,4-Trichloro	I-1	~1	1	†	III-1	~1	1	†
2,4,5-Trichloro	I-1	~1	1	†	III-1	~1	1	†
2-Bromo	I-1	2	2(2)	-2.6	III-1	~2	2(2)	†
	I-3	1	1§	+0.2				
3-Bromo	I-3	2	1	+0.3	III-1	~2	2(1)§	†
4-Bromo	I-1	2	2(2)	-2.5	III-1	~2	1	†
	I-3	1	1§	-0.3				
2,4-Dibromo	I-1	1	1	+1.4	III-1	1	1	-0.3
2-Iodo	I-1	2	2(2)	+0.9	III-1	2	2(2)	+0.2
4-Iodo	I-1	~2	2(2)	†	III-1	~2	2(2)	†

* See also "Experimental".

† Mean relative error greater than 3%.

§ Further bromine consumption after the break used for determination of end-point.

stituted fluoro- and chloroanilines without nuclear alkyl groups are best titrated in medium I-1 if they possess no *meta*-situated halogen atom, and in medium I-3 if they do have one. In the former case bromine enters all vacant *ortho* and *para* positions, in the latter case the number of hydrogen atoms exchanged is one less than the number of available *ortho* and *para* positions. However, for 2,3-dichloroaniline a further consumption of bromine after the end-point can be seen. If the haloaniline contains, in addition to a chlorine atom in the *meta*-position, a methyl group in the *ortho* or *para* position, the titration should be done in medium I-1. The same is valid for 3,5-dichloroaniline. While the methylanilines are fully substituted, only two of the three available positions in 3,5-dichloroaniline are taken up by bromine.

The considerable difference in reactivity-promoting properties between the I-1 and I-3 media for haloanilines is exemplified by the reactions of some chloroanilines with bromine in the two media. Thus, in 4-chloro-, 2,5-dichloro- and 2,4-dichloroaniline one hydrogen atom less is exchanged for bromine in medium I-3 than in medium I-1. This means that the last-mentioned compound does not react in medium I-3. For the two other compounds, only the best values are given in Table 2. As the two media differ only in the bromide concentration, we have here

another example of the marked influence of this factor on the reactivity for anilines, previously established for alkylanilines.¹ Other examples follow.

It has not been possible to brominate quantitatively the two trichloroanilines, 2,3,4- and 2,4,5-trichloroaniline, in any of the media used in this work. They do not react at all in medium I-3, and while approximately one mole of bromine is consumed per mole in medium I-1, the results are 5–10% low, and still lower values were obtained in medium III-1.

Bromoanilines react like their fluoro and chloro analogues. However, for 2- and 4-bromoaniline the reaction is somewhat sluggish in medium I-1, giving low results. The titration is better performed in medium I-3, where a useful break is obtained when one hydrogen atom is substituted.

Only two iodoanilines were titrated, namely 2- and 4-iodoaniline. Of these only the first could be brominated quantitatively, two bromine atoms entering the molecule when media I-1 and III-1 were used. It thus behaved like the other 2-halo-substituted anilines. 4-Iodoaniline reacted sluggishly and gave a sloping titration curve from which the end-point could not be accurately evaluated. The reason for this is not known, but it was observed that the electrodes were easily contaminated in this case. A similar behaviour was found for 2- and 4-iodophenol in a pre-

Table 3*. Results of bromination of anilines containing various oxygenated functional groups

Aniline	Titn. medium	Number of H substituted	Number of breaks in the titration curve	Mean relative error, %	Titn. medium	Number of H substituted	Number of breaks in the titration curve	Mean relative error, %
2-Methoxy	I-2	2	1	±0.0	III-1	2	1	+1.0
4-Methoxy	I-media	†	†	†	III-1	2	1	+0.8
2-Formyl	I-3	~1	1‡	§	III-1	1	2(1)‡	+1.5
3-Formyl	I-2	2	2(2)	-0.5	III-1	~2	1	§
<i>N,N</i> -Dimethyl-4-formyl	I-1	1	1	+1.7	III-1	~1	1	§
4-Acetyl	I-3	1	1	+1.5	III-1	~2	2(2)	§
4,4'-Bisdimethylaminobenzo-phenone	I-1	2	1	+1.3	III-1	1-2	1	§
2-Carboxy	I-3	2	2(2)	+0.9	III-1	2	1	-0.1
3-Carboxy	I-1	3	1	-1.0	III-1	~2	1	§
<i>N,N</i> -Dimethyl-3-carboxy	I-1	~1	1	§	III-1	1	1	±1.4
4-Carboxy	I-1	2-3	2(2)	§	III-1	2	2(2)	±0.6
2-Carbomethoxy	I-3	1	1‡	+1.5	III-1	~2	2(2)	§
4-Carboethoxy	I-3	1	1‡	+0.8	III-1	1-2	2(2)	§
4-Carboxy-methyl	I-1	2	1	-1.7	III-1	~2	1	§
2-Sulphonic acid	I-1	2	1	-0.5	III-1	2	1	-0.5
3-Sulphonic acid	I-1	2	1	-1.7	III-1	~2	1	§
4-Sulphonic acid	I-1	2	2(2)	-0.3	III-1	2	1	-0.1
2-Nitro	I-1	1	1	-0.9	III-1	~1	1	§
3-Nitro	I-2	~1	1‡	§	III-1	~1	1	§
4-Nitro	I-1	1	1	-0.4	III-1	1	1	-0.4
Dinitro-(2,4-, 2,6-, 3,5-)	I-media	0	0	—	III-1	0	0	—

* See also "Experimental".

† Not possible to determine from the titration curve.

§ Mean relative error greater than 3%.

‡ Further bromine consumption after the break used for determination of end-point.

vious investigation, neither compound being brominated quantitatively.⁶ The mean relative error for the haloanilines titrated, for the best medium for each compound, is $\pm 0.5\%$.

Anilines with various oxygenated functional groups

The anilines covered in this section contain methoxy, formyl, acetyl, carboxyl, ester, carboxymethyl (CH_2COOH), sulphonic acid and nitro groups in addition to the amino group. They are on the whole more difficult to determine than the haloanilines, which is also reflected in the results, which are less accurate than those obtained for the haloanilines (see Table 3). The reasons for this are *inter alia* that several of the anilines are sensitive to oxidation and are accordingly easily transformed during storage. Bromine can also oxidize the compounds, or displace some substituent groups such as formyl, carboxyl and sulphonic acid groups, causing overconsumption of titrant.⁴

Methoxyanilines. These anilines differ from the other compounds in Table 3 in having an activating functional group in addition to the amino group. Of the two compounds investigated, 2-methoxyaniline could be titrated without difficulty in pyridine-containing as well as in pyridine-free media, whereas 4-methoxyaniline produced a sloping titration curve in the former type of medium, from which the end-point could not be evaluated. The fact that the solution turned lilac indicated that some secondary reaction was taking place, probably oxidation. Bromine entered all free *ortho* and *para* positions.

Anilines with formyl and keto groups. With one exception these compounds are best titrated in pyridine-containing media of group I. The exception was 2-aminobenzaldehyde which gave a low value in these media. In medium III-1 two breaks were obtained in the titration curve. The first break corresponded to the substitution of one hydrogen atom by bromine and, although small, was usable for evaluation of the end point. The second break corresponded to the substitution of about 1.7 hydrogen atoms and was not useful for quantitative analysis.

With the exception of this compound, the number of hydrogen atoms exchanged in each aromatic ring was one less than the number of available *ortho* and *para* positions.

Anilines with carboxyl and ester groups. For the quantitative bromination of these groups of substituted anilines, pyridine-containing as well as pyridine-free media are necessary. Thus, 4-carboxyaniline and *N,N*-dimethyl-3-carboxyaniline could only be titrated in the pyridine-free medium III-1. For these compounds there is a considerable overconsumption of bromine in pyridine-containing media, which for 4-carboxyaniline is due to partial displacement of the carboxyl group by bromine. Delgado⁴ has demonstrated that the decarboxylation of *p*-aminobenzoic acid on bromination in aqueous media is a function of pH. Decarboxylation increases when the pH is

raised and becomes quantitative at pH 5. Obviously, the presence of pyridine in medium I-1 raises the pH enough to cause a partial displacement of the carboxyl group by bromine during the titration, as the values are 15–20% too high. In the absence of pyridine, in medium III-1, no displacement of the carboxyl group occurs.

Delgado reported a similar decarboxylation for *o*-aminobenzoic acid in aqueous media at pH 3–5, while in this work only a slight overconsumption of bromine was observed for this compound in the pyridine-containing medium I-3. It is of interest to compare the results obtained for 3-carboxyaniline and its *N,N*-dimethyl derivative. The presence of the two methyl groups at the nitrogen atom decreases the number of bromine atoms entering the ring, from three to one in medium I-1 and from two to one in medium III-1. Similar results were previously observed for the corresponding 3-methylanilines where the decrease in medium I-1 was from three to two and in medium III-1 from three to one.¹

The two anilines substituted by ester groups, 2-carbomethoxy- and 4-carboethoxyaniline, should be titrated in medium I-3 where the end-point corresponds to entry of one bromine atom into the molecule. However, a further consumption of bromine is evident from the appearance of the titration curve.

Anilines with sulphonic acid and nitro groups. Anilines with sulphonic acid groups in the *ortho* or *para* position can be titrated in pyridine-containing (I-1) as well as pyridine-free (III-1) media with good accuracy while, for metanilic acid, pyridine must be present in the medium if quantitative results are to be obtained. In view of the deactivation exerted by the sulphonic acid group it is somewhat surprising to find that in the 2- and 4-amino sulphonic acids all free *ortho* and *para* positions are substituted by bromine in both pyridine-containing and pyridine-free media. In the corresponding *meta* isomer two of the three vacant positions are substituted.

Nitroanilines are strongly deactivated and not very prone to react with bromine. Thus, of the six compounds tested only 2- and 4-nitroaniline gave quantitative results, the three dinitroanilines did not react, and 3-nitroaniline failed to react quantitatively. For the mononitroanilines only one bromine atom entered the aromatic ring.

In order to facilitate the correct choice of titration medium for the quantitative bromometric titration of substituted anilines, Table 4 has been included. In cases where two media give results of comparable accuracy, the second best medium has been put in parentheses.

Comparison of reactivity of various kinds of anilines with bromine

It is of interest to compare the reactivity towards bromine, in the same medium, of various types of anilines studied in this and in a previous investigation.¹ Table 5 gives the substituent group and the

Table 4*. Media for quantitative bromination of substituted anilines.

Functional group	Medium	Functional group	Medium
Halogen (2- or 4-)		Methoxy (2-)	I-2
F	I-1	Methoxy (4-)	III-1
Cl	I-1	Formyl (2-)	III-1
Br	I-3	Formyl (3-)	I-2
I (2-)	III-1	Acetyl (4-)	I-3
Halogen (3-)		Carboxyl (2-)	II-1
F	I-3	Carboxyl (3-)	I-1
Cl	I-3 (III-1)	Carboxyl (4-)	III-1
Br	I-3	Ester (2-)	I-3
Halogen (di)		Ester (4-)	I-3
Cl (2,3-)	I-3	Carboxymethyl (4-)	I-1
F (2,4-)	I-1	Sulphonic (2-)	I-1 (III-1)
Cl (2,4-)	I-1	Sulphonic (3-)	I-1
Br (2,4-)	III-1	Sulphonic (4-)	III-1 (I-1)
F (2,5-)	I-3	Nitro (2-)	I-1
Cl (2,5-)	III-1 (I-3)	Nitro (4-)	I-1 (III-1)
Cl (2,6-)	III-1 (I-1)		
Cl (3,4-)	I-3		
Cl (3,5-)	III-1 (I-1)		

* See also text.

number of bromine atoms entering the molecule, when the bromination is performed in the pyridine-free medium III-1 containing 60% v/v of acetic acid. Certain conclusions can be drawn from this table.

When the substituent is in the 2- or 4-position relative to the amino group in a primary aniline there is no great difference in reactivity between compounds with activating (Me or MeO) and deactivating groups, hydrogen at the free *ortho* and *para* positions being exchanged for bromine, with some exceptions. Thus for anilines with certain deactivating groups, *i.e.*, formyl and nitro groups, the number of hydrogen atoms exchanged falls to one. Another factor which can decrease the number of entering bromine atoms is the presence of alkyl groups at the nitrogen atom (secondary and tertiary anilines). The size and number

of alkyl groups at the nitrogen atom control the number of entering bromine atoms.

When the substituent group is situated in the 3-position there is a difference between anilines with activating (Me) and deactivating groups. In the former case bromine enters all free *ortho* and *para* positions (3), in the latter, one less (2). The reason for this should be sought in the fact that a *meta* methyl group is in a position to activate all three vacant *ortho* and *para* positions. An exception is constituted by 3-nitroaniline where only one bromine enters. As with 2- and 4-substituted anilines, the reactivity of the 3-substituted anilines is reduced by the introduction of alkyl groups at the nitrogen atom.

The behaviour of the anilines in the substitution reaction with bromine in medium III-1 should be compared with the analogous reaction between phenols and bromine in the same medium. In this case only one bromine atom enters the ring of a 2-, 3-, or 4-substituted alkylphenol and no reaction takes place if the substituent is deactivating.

CONCLUSIONS

It is concluded that substituted anilines containing halogen or various oxygenated functional groups can be titrated quantitatively with coulometrically generated bromine in water-acetic acid media, with or without added pyridine. The standard titration media are the pyridine-containing media of group I, but in certain instances the pyridine-free medium III-1 should be used. Not all of the investigated anilines can be determined. Thus, trichloroanilines, *p*-iodoaniline and *m*-nitroaniline, although reactive, failed to give quantitative results, while dinitroanilines did not react.

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Table 5. Comparison between reactivity towards bromine for various kinds of anilines in medium III-1

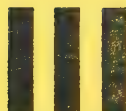
Substituent group	Number of H substituted	Substituent group	Number of H substituted	Substituent group	Number of H substituted
2-Me	2	3-Me	3	4-Me	2
<i>N</i> -Me-2-Me	2	<i>N</i> -Me-3-Me	2	<i>N</i> -Me-4-Me	2
<i>N</i> -Et-2-Me	1	<i>N</i> -Et-3-Me	2	<i>N</i> -Et-4-Me	0
<i>N,N</i> -Me ₂ -2-Me	0	<i>N,N</i> -Me ₂ -3-Me	1	<i>N,N</i> -Me ₂ -4-Me	0
<i>N,N</i> -Et ₂ -2-Me	0	<i>N,N</i> -Et ₂ -3-Me	1	<i>N,N</i> -Et ₂ -4-Me	0
2-MeO	2	3-F	2	4-MeO	2
2-F	2	3Cl	2*	4-F	1-2
2-Cl	2	3-Br	~ 2*	4-Cl	~ 2
2-Br	2	3CHO	~ 2	4-Br	~ 2
2-I	2	3-COOH	~ 2	4-I	~ 2
2-CHO	1*	<i>N,N</i> -Me ₂ -3-COOH	1	<i>N,N</i> -Me ₂ -4-CHO	0-1
—	—	3-SO ₃ H	~ 2	4-COCH ₃	~ 2
2-COOH	2	3-NO ₂	~ 1	4-COOH	2
2-COOMe	~ 2			4-COOEt	1-2
2-SO ₃ H	2			4-SO ₃ H	2
2-NO ₂	~ 1			4-NO ₂	1

* Further bromine consumption can be noticed.

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STUDY OF RETENTION BEHAVIOUR OF PRIMARY, SECONDARY AND TERTIARY ANILINES IN STRAIGHT- AND REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

A series of alkyl-substituted prim., sec. and tert. anilines were chromatographed on three liquid chromatographic systems: one straight-phase system on nitrile phase and two reversed-phase systems on octadecyl- and octylsilane, respectively. On the nitrile phase the elution order is tert., sec. and prim. anilines and within each group the retention is mainly determined by the base strength, the number and size of ortho-situated alkyl groups and by the size of the alkyl groups substituted at the nitrogen atom. On the reversed phases the anilines within each group are mainly eluted after increasing alkyl carbon number.

The relationship between pK_b of the anilines and $\lg k'$ on the nitrile phase is discussed and a comparison is made between k' for prim. anilines and the corresponding phenols on the same phase. Identification of different kinds of anilines by means of a two-phase plot is discussed and a method described for the systematic separation of alkyanilines into structure classes and individual compounds.

INTRODUCTION

In a previous paper from this laboratory liquid chromatography (LC) was evaluated for the analysis of a series of alkyl-substituted phenols and some useful relationships established between structure and elution order on various phases¹. As an extension of this work a similar study of alkyanilines is presented in this paper. It involves some 40 alkyl-anilines, including prim., sec. and tert. anilines, which were run on straight- and reversed-phase systems.

It seems, that up to now very little attention has been paid to bonded phase LC assay of this kind of amines, in spite of the fact that they are widely used in the chemical industry and that their toxicity makes it essential to be able to control the occurrence of them in the environment. Thus, in the first successful application of a bonded stationary phase to LC analysis of anilines, some phenylene diamines were separated on an "ether"-bonded phase².

It was concluded that this phase in combination with cyclopentane/methanol as carrier was useful for the separation of compounds containing N-H groups. Later on, Sleight³ made a systematic survey of the elution of some phenols and a few alkylanilines, from some commercial bonded phases, Durapak OPN and Durapak Carbowax 400. However, these phases are today obsolete for bonded phase LC. Recently, Frohlinger *et al.*⁴ reported the separation and quantitation of some aromatic amines occurring in the working environment by means of ion exchange chromatography, using a surface sulfonated cation exchanger and perchloric acid as the eluent.

EXPERIMENTAL

Apparatus

The LC pump used was a Varian Model 4100 (Varian, Palo Alto, Calif., U.S.A.) and the detector was a Laboratory Data Control Model 1285 UV monitor (Laboratory Data Control, Riviera Beach, Fla., U.S.A.) used at 280 nm. Sample application was accomplished by a valve injector (Rheodyne, Berkeley, Calif., U.S.A.) with a 20 μ l loop.

Columns

The bonded-phase packing materials were the commercially available LiChrosorb RP8, 10 μ m (E. Merck, Darmstadt, G.F.R.), Nucleosil C₁₈, 5 μ m and Nucleosil CN, 5 μ m (Marchery-Nagel & Co., Düren, G.F.R.). LiChrosorb RP8 was packed with the balanced density technique using tetrabromoethane. Nucleosil C₁₈ and Nucleosil CN were packed in accordance with the upward-slurry packing technique^{5,6}. All columns consisted of precision-bore stainless steel tubing (4.4 mm I.D. $\times$ 200 mm). The columns were used at room temperature.

For accurate work it is necessary to reactivate the columns, especially the nitrile column, regularly. This was made according to recommended procedures. The stability of the columns was tested daily using a mixture of phenol, 2,6-dimethylphenol and 4-tert.butylphenol for the reversed-phases and a mixture of diphenylamine, triphenylamine and N-methyl-2-methylaniline for the nitrile phase.

Chemicals

Isooctane (certified A.C.S. grade, Fischer Scientific, Fairlawn, N.J., U.S.A.), methanol (analytical-reagent grade, May & Baker, Dagenham, Great Britain), 2-propanol (pro analysi grade, E. Merck, Darmstadt, G.F.R.), sodium dihydrogen phosphate, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (99%, BDH Chemicals, Poole, Great Britain), disodium hydrogen phosphate, $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (according to Sørensen, E. Merck) and orthophosphoric acid (pro analysi grade, E. Merck) were used for preparing the LC eluents.

The anilines were of the best grade commercially available. Some of them were further purified by distillation or recrystallization. N,N-dimethyl-2,6-dimethylaniline, N,N-diethyl-2-ethylaniline and N-ethyl-2-ethylaniline were prepared at this laboratory.

Mobile phases

For reversed-phase LC on C_8 and C_{18} phases methanol-aqueous buffer was used and for straight-phase LC on the nitrile phase isooctane, containing 0.2% (v/v) 2-propanol, was applied.

Methanol-aqueous buffer (60:40, v/v, pH = 7.0). Fifteen ml of 0.025 mole l^{-1} Na_2HPO_4 + 250 ml of 0.025 mole l^{-1} NaH_2PO_4 + 397.5 ml of methanol; pH was adjusted to 7.0 with small amounts of orthophosphoric acid and dilute (1 mole l^{-1}) sodium hydroxide.

Methanol-aqueous buffer (70:30, v/v, pH = 7.0). Fifteen ml of 0.025 mole l^{-1} Na_2HPO_4 + 250 ml of 0.025 mole l^{-1} NaH_2PO_4 + 618.33 ml of methanol; pH was adjusted as above.

Methanol-aqueous buffer (80:20, v/v, pH = 7.0). Fifteen ml of 0.05 mole l^{-1} Na_2HPO_4 + 250 ml of 0.05 mole l^{-1} NaH_2PO_4 + 1060 ml of methanol; pH was adjusted as above.

Procedure

Anilines were dissolved in methanol or isooctane to give concentrations of approximately $0.02\text{--}0.04\text{ mg}\cdot\text{ml}^{-1}$ and 20 μl of the solutions were injected on to the LC columns. The capacity factor given is a mean value from at least three injections with a relative standard deviation of about 3%. Retention times of unretained solutes were determined by injecting n-hexane on the nitrile phase and sodium nitrate solution (0.05%, w/w), on the reversed-phases. The capacity factor, k' , was calculated from the following formula

$$k' = \frac{t_R - t_o}{t_o} \quad (1)$$

where t_R = retention time of sample,

t_o = retention time of unretained solute.

The systematic separation procedure described later on involves the class separation of prim., sec. and tert. anilines on the nitrile phase, followed by an alkyl carbon number separation on the C_{18} phase. The fractions of anilines in isooctane solution, collected from the nitrile phase, cannot be directly introduced on to the C_{18} phase, but have to be transferred to methanol before injection. About 200 μl of methanol were added and the mixture shaken in order to extract the anilines. The methanol solution was then injected on to the C_{18} phase.

Acetylation procedure for the separation of sec. and tert. anilines

The mixture of sec. and tert. anilines, eluted from the nitrile phase with isooctane, was collected in a test tube. The content was reduced to about 1/4 of its original volume by blowing a gentle stream of nitrogen over the surface at room temperature. One ml of acetic anhydride was added and the mixture heated over a small flame to near boiling for a minute. The excess of acetic anhydride was then removed by a nitrogen stream as above to near dryness.

In order to separate the tert. anilines from the acetylated secondary, ca. 100 μl of isooctane were added and the solution injected on top of a short glass column (50 mm $\times$ 4 mm), filled with nitrile phase (slurry packed without pressure). Tert. anilines were eluted with 2-3 ml of isooctane and then, acetylated sec. anilines by the same volume of ethanol, and separately collected.

The tert. aniline fraction can be injected on to the nitrile phase after concentration, while the acetylated sec. anilines have to be hydrolyzed first. Two ml of 50% (v/v) sulphuric acid were added and the mixture heated to near boiling for 10 min. After cooling, the acid was neutralized with dilute sodium hydroxide in some excess and the free sec. anilines extracted with a few ml of isooctane. After concentration with a nitrogen stream as before, the solution is ready for injection on to the nitrile phase.

RESULTS AND DISCUSSION

The liquid chromatographic investigation comprises three chemically bonded phases; one straight-phase system on nitrile phase and two reversed-phase systems on octyl- and octadecylsilane, respectively. In the latter case, two eluents with different proportions of methanol-aqueous buffer were investigated.

Straight-phase LC

The results of the runs on the nitrile phase are given in Table I. The anilines are arranged in the order of prim., sec. and tert. anilines. Within the primary group of anilines the compounds are placed in order of increasing number of alkyl groups, substituted at the benzene ring, and within the groups of sec. and tert. anilines, in order of increasing size of substituents at the nitrogen atom.

It can be seen from this Table and from Fig. 7 that prim. anilines travel more slowly on the nitrile phase than any of the sec. and tert. anilines investigated, and secondary ones more slowly than tertiary, with some exceptions. This fact indicates that the migration is at first hand governed by the ease of access to the nitrogen atom. This conclusion is further confirmed by an examination of the order of elution within each group of anilines.

For prim. anilines, i.e. anilines without alkyl groups at the nitrogen atom, the elution order is mainly determined by the number and size of ortho-situated alkyl groups. Thus, prim. anilines with two ortho-situated alkyl groups have the lowest retention, and those without such groups, the highest. Among the latter, para-substituted anilines travel more slowly than the corresponding meta-substituted and the size of the alkyl group seems to be of minor importance for the retention. This fact is demonstra-

ted by the series of 4-alkyl-substituted prim. anilines investigated.

The difference in retention between para-substituted prim. anilines without ortho-substituents and the corresponding meta-substituted, can be ascribed to the fact that anilines belonging to the former group are stronger bases and accordingly more ionized in the eluent than the members in the latter group.

The influence of ortho-substitution on the interaction of prim. anilines with the nitrile phase is demonstrated in Fig. 1, where $\lg k'$ was plotted against the alkyl carbon number. It can be seen that the anilines arrange themselves into three groups according to the number of ortho-situated substituents, viz. non-ortho-, mono-ortho- and di-ortho-substituted compounds, divided by dashed lines in Fig. 1. In a previous study the same relationship was found to apply to phenols¹.

For sec. anilines the retention on the nitrile phase is mainly governed by two factors, viz. by the size of the alkyl group substituted at the nitrogen atom and by the presence or absence of ortho-situated alkyl groups and by their size. Thus, $\lg k'$ decreases linearly with N-alkyl carbon number for N-alkylanilines without nuclear substituents (N-alkyl = methyl to butyl, Nos. 18, 22, 29, 30 in Fig. 1), and a further decrease then results from ortho-substitution of hydrogen against alkyl groups. Just as for prim. anilines, the effect of meta-substitution on retention is slight while para-substitution increases retention.

For the retention of tert. anilines on the nitrile phase the same rules apply as for secondary. Accordingly, retention is a function of the size of the alkyl groups at the nitrogen atom, decreasing with increasing size. In the same way ortho-substitution of alkyl groups decreases retention. Among tert. anilines, those with one or two ortho-situated alkyl groups can be clearly distinguished from sec. anilines by their lower retention, while there occurs some mixing between other kinds of tert. anilines and secondary (Fig. 1).

Relationship between pK_b of anilines and $\lg k'$ on the nitrile phase

It is interesting to note that if $\lg k'$ on the nitrile phase is plotted against pK_b of the prim. anilines^{7,9}, a semi-linear correlation is obtained (Fig. 2). A similar relationship between pK_b and $\lg k'$ on silicagel was reported to exist for toluidines and phenylene diamines,

respectively¹⁰. Among sec. anilines, N-methylanilines form a group of their own, while N-alkylanilines with ethyl and larger alkyl groups align themselves around a lower line.

To this group also belong some tert. anilines viz. N,N-dimethylanilines without ortho-situated alkyl groups. For tert. anilines with greater steric hindrance around the nitrogen atom, which appear at the bottom of the plot, the spread of points is considerable. This group comprises N,N-dimethylanilines with ortho-situated methyl groups and N,N-diethylanilines.

Within each of the above-mentioned groups of anilines there is a general trend of increasing k' values with decreasing pK_b values, i.e. with increasing base strength of the compounds. This would be expected on the conditions that the pK_b value is a measure of the ability of the amino group to accept a proton and the straight-phase k' value mainly is a measure of its ability to interact with a nitrile group in the bonded phase.

Because of the different steric requirements of the proton and the nitrile group the semi-linear relationship between pK_b and $\lg k'$ is only valid for sterically equivalent anilines, which fact causes a subdivision into structure groups as pictured in Fig. 2. Accordingly, the k' value of an alkyaniline on the nitrile phase system is primarily a function of its base strength and of the substitution pattern around the nitrogen atom; the latter mainly determining the subdivision into structure groups, whereas the position of an aniline within a group is governed by the base strength as well as steric factors.

Reversed-phase LC

Three systems were studied, two on C_{18} phase with methanol-aqueous buffer (80:20 and 70:30, v/v, respectively), as eluents and one on C_8 phase with methanol-aqueous buffer (60:40, v/v) as eluent. In all cases pH was adjusted to 7.0. The results are collected in Table I. It is evident, that for prim. anilines the alkyl carbon number determines the retention while, the position of the alkyl groups in the benzene ring is of minor importance.

When moving alkyl groups from the nucleus to the nitrogen atom a distinct increase in retention occurs. Thus, sec. anilines invariably have higher retentions than primary with the same total alkyl carbon num-

ber, and the same applies to tert. anilines vs. secondary. Accordingly, if retention on the C_{18} phase is plotted against total alkyl carbon number, prim., sec. and tert. anilines align themselves along three parallel lines (Fig. 3).

Of the two solvent systems used for the C_{18} phase, the 80:20 eluent elutes lower prim. anilines too rapidly for a good resolution to be obtained, and the 70:30 eluent elutes higher tert. anilines too slowly to be of practical value. A compromise between the two solvent systems should therefore best meet the case of a more universal isocratic solvent for alkyranilines on the C_{18} phase.

The C_8 phase was investigated using methanol-aqueous buffer (60:40, v/v, pH = 7.0) as eluent. On this system alkyranilines behave much as on the C_{18} phase with the 70:30 eluent, the retentions being slightly higher (Table I).

Comparison between prim. anilines and phenols

Prim. anilines are structurally analogous to phenols and it is, accordingly not surprising to find their behaviour in LC to be similar. For phenols it was concluded that differences in strength of hydrogen bonding to the proton-accepting cyano group was responsible for the division into non-ortho-, mono-ortho- and di-ortho-substituted compounds, when run on the nitrile phase¹. The same explanation should be valid for the corresponding prim. anilines, although the division into structure classes is less sharp in this case.

In Fig. 4, nitrile phase k' values of alkylphenols from ref. 1 have been plotted against nitrile phase k' values of corresponding prim. anilines, taken from Table I. As can be seen, there is a considerable spread of points indicating different behaviour of prim. anilines and phenols on the nitrile phase. Although the two nitrile phases used are different, it is felt that certain conclusions may be drawn from the figure.

The points are more or less aligned along two straight lines. Along the upper line are mainly found di-ortho-alkyl-substituted compounds and compounds having a para-situated alkyl group. For these compounds there is no great difference between k' values of corresponding anilines and phenols. This can in the former case be ascribed to the fact that the ortho-situated alkyl groups partly inhibit hydrogen bonding, making the chromatographic properties of the aniline and the phenol more similar. A levelling effect on the hydrogen bonding properties would also be exerted by a para-substituted alkyl group, which tends to weaken the acid properties of phenols

and strengthen the basic properties of anilines⁷.

Along the lower line, are mainly compounds with a single ortho- or meta-situated alkyl group. For these compounds there is a considerable difference between k' values for anilines and corresponding phenols. In this instance the levelling effect of the alkyl groups is smaller, and the stronger hydrogen bonding of the phenolic hydroxyl group causes phenols to move slower than corresponding anilines on the nitrile phase. This difference is most pronounced for phenol and aniline themselves and, as can be seen from Fig. 4, their point lying far outside the two regions discussed above.

In reversed-phase chromatography of prim. anilines and phenols the influence of the two functional groups is small, retention being mainly determined by the number of alkyl groups. This fact is demonstrated in Fig. 5 where k' values on the C_{18} phase for corresponding compounds have been plotted. The slightly higher k' values of anilines on this phase can be ascribed to the presence of residual acidic silanol groups in the C_{18} phase, which interact with the basic amino groups, and to the fact that phenols are stronger electrolytes.

Use of two-phase plot for the identification of alkylanilines

In Fig. 6, $\lg k'$ for the C_{18} phase system has been plotted against $\lg k'$ for the nitrile phase system. It appears that the plot may be divided into zones for different kinds of anilines. Thus, all prim. anilines fall to the right of vertical line 1 (zone P), and the horizontal line divides the sec. and tert. anilines into two zones below and above the line (zones S and T, respectively). The P-zone may in turn be divided by two vertical lines into zones for non-ortho-substituted compounds (to the right of line 3), mono-ortho-substituted compounds (between line 2 and 3) and di-ortho-substituted compounds (between line 1 and 2).

Although the boundaries between some of the zones are rather narrow and some mixing of compounds occurs, it is felt, that the diagram in Fig. 6 would be useful for identification purposes. The fact that different kinds of alkylanilines fall into different zones will make it possible to distinguish to a great extent between prim., sec. and tert. alkylanilines and between the three structural classes of prim. anilines. In addition, the zone diagram gives information about alkyl carbon number, anilines with the lowest number appearing at the bottom of each zone and those with the highest number at the top.

There is just one tert. aniline falling into the S-zone; as it is the lowest member in the series of tert. anilines, viz. N,N-dimethylaniline, it is safe to conclude that no other tert. aniline will appear in this zone. The T-zone contains some sec. anilines. This is unavoidable, since the horizontal separation between sec. and tert. anilines, i.e. the separation on the nitrile phase, is insufficient and the vertical position of a sec. aniline in the diagram is determined solely by the alkyl carbon number.

It can be concluded, that it is impossible to decide whether an aniline falling into the T-zone is in fact a secondary one. For this purpose other methods must be resorted to. A simple test to distinguish between the two classes of amines, is to heat the sample for a couple of minutes with an excess of acetic anhydride and then run the product on the nitrile phase system. If no change of retention is observed, the aniline is tertiary and, if no peak is obtained, or the retention time is much increased, the amine is secondary. The reason for this behaviour is that only sec. anilines react with acetic anhydride and, that the acetylated amine travels very slowly on the nitrile column (c.f. below).

Systematic separation of alkyilanilines into prim., sec. and tert. anilines and individual compounds

As pointed out previously, the investigated prim. alkyilanilines may be separated as a class from sec. and tert. anilines on the nitrile phase, and the latter two classes also largely resolved on the same phase. Accordingly, the problem of separating an unknown mixture of alkyilanilines by LC should be solved by first running the mixture on the nitrile phase, isolating the compounds according to class, and then running the three classes separately on the C_{18} phase, where a separation according to alkyl carbon number is achieved. When this procedure was applied to the 41 anilines (Nos. 1-26 and 29-43) collected in Table I, the chromatograms shown in Figs. 7-8 were obtained.

If the contents in the peaks representing the different carbon numbers are collected from the C_{18} phase and rerun on the nitrile phase, the peaks are largely resolved into individual compounds as demonstrated in Figs. 9-10. In order to distinguish between prim., sec. and tert. anilines, 2,6-dimethylaniline (No. 15) and N-propylaniline (No. 29) are admixed for marking the boundaries between the three structure classes.

In Fig. 8 the tert. aniline fraction has been run as obtained from the nitrile phase which means that it contains some sec. anilines. However, for mixtures of unknown composition it is recommended, first to separate the two groups of anilines by the acetylation method described below, and then run them separately on the C_{18} phase.

The acetylation procedure involves, as detailed in the experimental part, treatment of the mixture of sec. and tert. anilines with acetic anhydride in order to convert the sec. anilines into acetyl derivatives. The mixture is then passed through a short column, packed with nitrile phase. Unchanged tert. anilines are rapidly eluted with isooctane, while acetylated sec. anilines travel more slowly and are eluted in a second step by a stronger solvent, viz. ethanol. On hydrolysis of the latter mixture, free sec. anilines result.

The application of the acetylation procedure to a mixture of eight sec. and tert. anilines is demonstrated in Fig. 11. This mixture contains all of the four sec. anilines (Nos. 23,26,29,30) which fall into the tertiary group in the original class separation on the nitrile phase (Fig. 8). In Fig. 11 (S+T) the chromatogram of the total mixture on the nitrile phase is given. After acetylation, separation and hydrolysis, the two chromatograms in T and S for tert. and sec. anilines, respectively, result, likewise on the nitrile phase. As can be seen, the class separation achieved is complete. The separation procedure outlined above will permit the class separation of complex mixtures of prim., sec. and tert. anilines and will also largely resolve the mixtures into individual compounds. In addition, information is obtained about alkyl carbon number and, to a certain extent, about the number and position of nuclear alkyl groups.

As shown by the chromatograms in Fig. 8, the investigated prim. and sec. anilines are eluted from the C_{18} phase in order of increasing alkyl carbon number. For tert. anilines there is some deviation from this rule as N,N-dimethyl-2,6-dimethylaniline (No. 37) with alkyl carbon number four appears after the three tert. anilines Nos. 39-41 with alkyl carbon number five. It seems, that a high degree of steric hindrance around the nitrogen atom will increase the retention on the C_{18} phase, resulting in a disturbance of the regular elution after alkyl carbon number.

N-Phenyl- and N-benzylanilines

The investigated material contains some anilines with aromatic groups substituted at the amino group. These anilines appear together with other sec. and tert. anilines, respectively, on the nitrile phase class separation (Fig. 7). On the following C_{18} phase carbon number separation, they are eluted last of the investigated compounds in their respective groups (Fig. 8). In the two-phase plot in Fig. 6 these compounds would appear in the T-zone and cannot be distinguished from other kinds of sec. and tert. anilines.

CONCLUSIONS

Bonded phase straight- and reversed-phase LC, using a nitrile phase and a C_{18} phase, can be applied for the separation of complex mixtures of alkyanilines and for the identification of individual compounds. This circumstance is based on the fact that the separation on the nitrile phase proceeds in the order tert., sec. and prim. anilines, which groups then can be resolved on the C_{18} phase after increasing alkyl carbon number.

For confirming the structure of an alkyaniline, several useful relationships are available viz. two-phase plots, correlations between $\lg k'$ and carbon numbers as well as between $\lg k'$ and pK_b of the anilines. By these means it is possible to ascertain the identity of an alkyaniline as to class and general structure with a fair degree of certainty.

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TABLE I

CAPACITY FACTORS, k' , FOR ALKYLANILINES IN FOUR LC SYSTEMS

Straight-phase system: Nucleosil CN. Mobile phase: 0.2% (v/v) 2-propanol in isooctane. Reversed-phase systems: (a) Nucleosil C₁₈. Mobile phases: methanol-aqueous buffer (80:20 and 70:30, v/v, pH = 7.0). (b) LiChrosorb RP8. Mobile phase: methanol-aqueous buffer (60:40, v/v, pH = 7.0).

No.	Aniline (Substituent)	k'			
			Nitrile phase	C ₁₈ phase Eluent (80:20) Eluent (70:30)	C ₈ phase Eluent (60:40)
<i>Primary anilines</i>					
1	None	7.53	0.41	0.55	0.60
2	2-Methyl	4.84	0.55	0.85	0.92
3	2-Ethyl	3.65	0.71	1.20	1.40
4	2-Isopropyl	3.14	0.86	1.63	2.07
5	3-Methyl	8.07	0.51	0.83	0.94
6	3-Ethyl	7.02	0.67	1.20	1.49
7	4-Methyl	9.62	0.54	0.85	0.97
8	4-Ethyl	8.72	0.71	1.26	1.59
9	4-Isopropyl	8.87	0.90	1.75	2.46
10	4- <u>n</u> -Butyl	8.72	1.38	3.11	4.84
11	2,3-Dimethyl	5.48	0.74	1.18	1.31
12	2,4-Dimethyl	6.04	0.78	1.25	1.49
13	2-Methyl-4- <u>n</u> -butyl	5.07	1.91	4.71	7.40
14	2,5-Dimethyl	4.45	0.75	1.26	1.43
15	2,6-Dimethyl	2.83	0.78	1.31	1.43
16	3,4-Dimethyl	9.51	0.69	1.16	1.40
17	2,4,6-Trimethyl	2.96	1.08	1.99	2.34
<i>Secondary anilines</i>					
18	N-Methyl	2.32	0.68	1.12	1.22
19	N-Methyl-2-methyl	1.43	0.97	1.68	1.86
20	N-Methyl-3-methyl	2.16	0.89	1.62	1.86
21	N-Methyl-4-methyl	2.73	0.90	1.63	1.93

Continued

TABLE I continued

22	N-Ethyl	1.49	0.87	1.54	1.77
23	N-Ethyl-2-methyl	0.82	1.34	2.57	2.91
24	N-Ethyl-3-methyl	1.40	1.14	2.26	2.71
25	N-Ethyl-4-methyl	1.72	1.15	2.26	2.73
26	N-Ethyl-2-ethyl	0.67	1.86	3.80	4.61
27	N-Acetyl		0.41	0.62	0.71
28	N-Acetyl-4-methyl		0.54	0.87	1.09
29	N-Propyl	1.06	1.23	2.35	3.00
30	N- <u>n</u> -Butyl	0.90	1.69	3.70	5.04
31	N-Phenyl	2.50	1.52	3.45	4.86
32	N-Benzyl	1.65	1.50	3.42	4.79
<i>Tertiary anilines</i>					
33	N,N-Dimethyl	0.65	1.35	2.42	2.68
34	N,N-Dimethyl-2-methyl	0.45	1.76	3.48	3.99
35	N,N-Dimethyl-3-methyl	0.68	1.82	3.61	4.17
36	N,N-Dimethyl-4-methyl	0.79	1.80	3.64	4.17
37	N,N-Dimethyl-2,6- -dimethyl	0.14	4.20	10.1	
38	N,N-Diethyl	0.50	2.32	5.21	6.44
39	N,N-Diethyl-2-methyl	0.29	3.45	7.71	8.89
40	N,N-Diethyl-3-methyl	0.54	3.08	7.31	9.18
41	N,N-Diethyl-4-methyl	0.90	2.95	6.81	8.06
42	N,N-Diethyl-2-ethyl	0.21	5.21	12.8	16.8
43	N,N-Diphenyl	0.39	7.40	25.9	

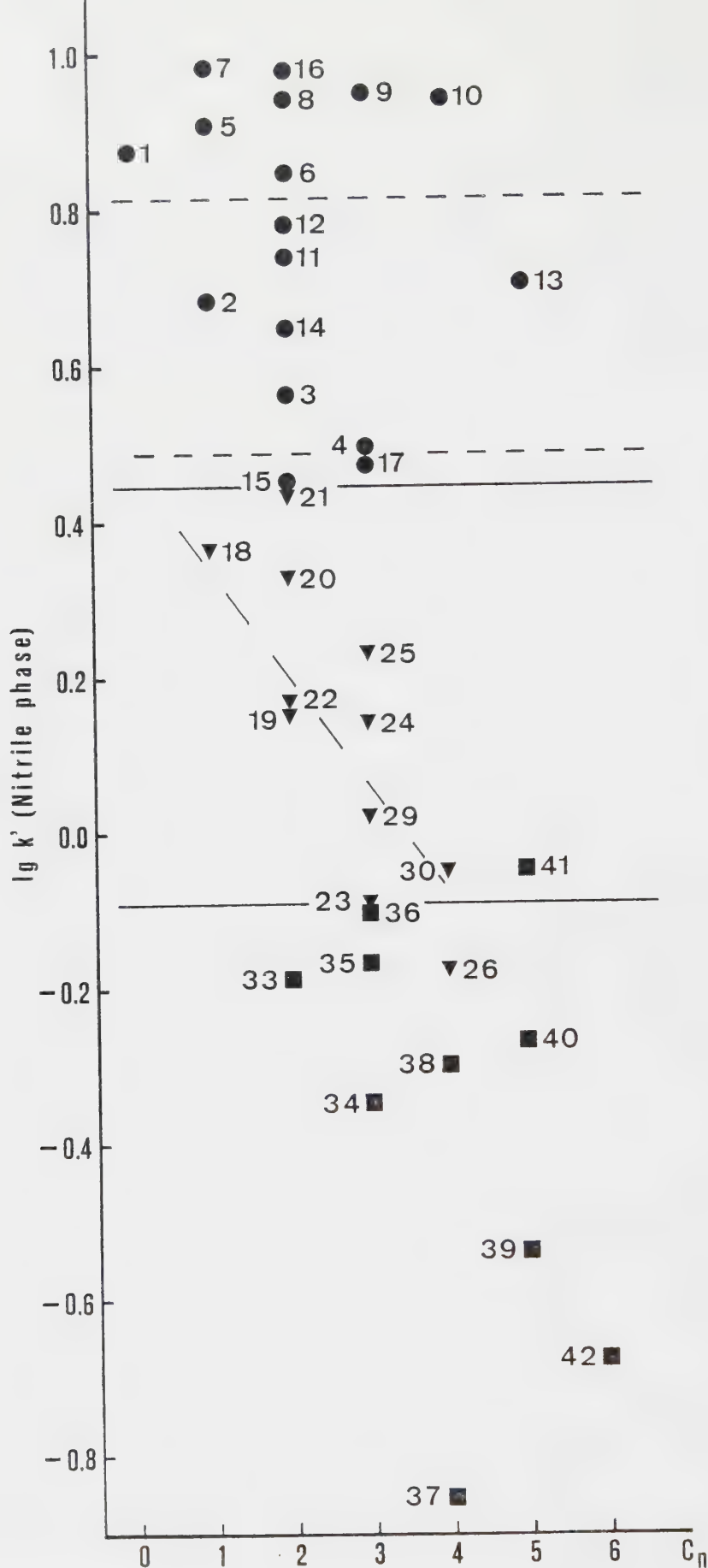


Fig. 1. Relationship between alkyl carbon number (C_n) and $\lg k'$ of alkyl-anilines on the nitrile phase. Stationary phase, Nucleosil CN; mobile phase, 0.2% (v/v) 2-propanol in isooctane; $45 \text{ ml} \cdot \text{h}^{-1}$. $\bullet$ = Prim. anilines; ∇ = sec. anilines; $\blacksquare$ = tert. anilines. The numbers refer to Table I.

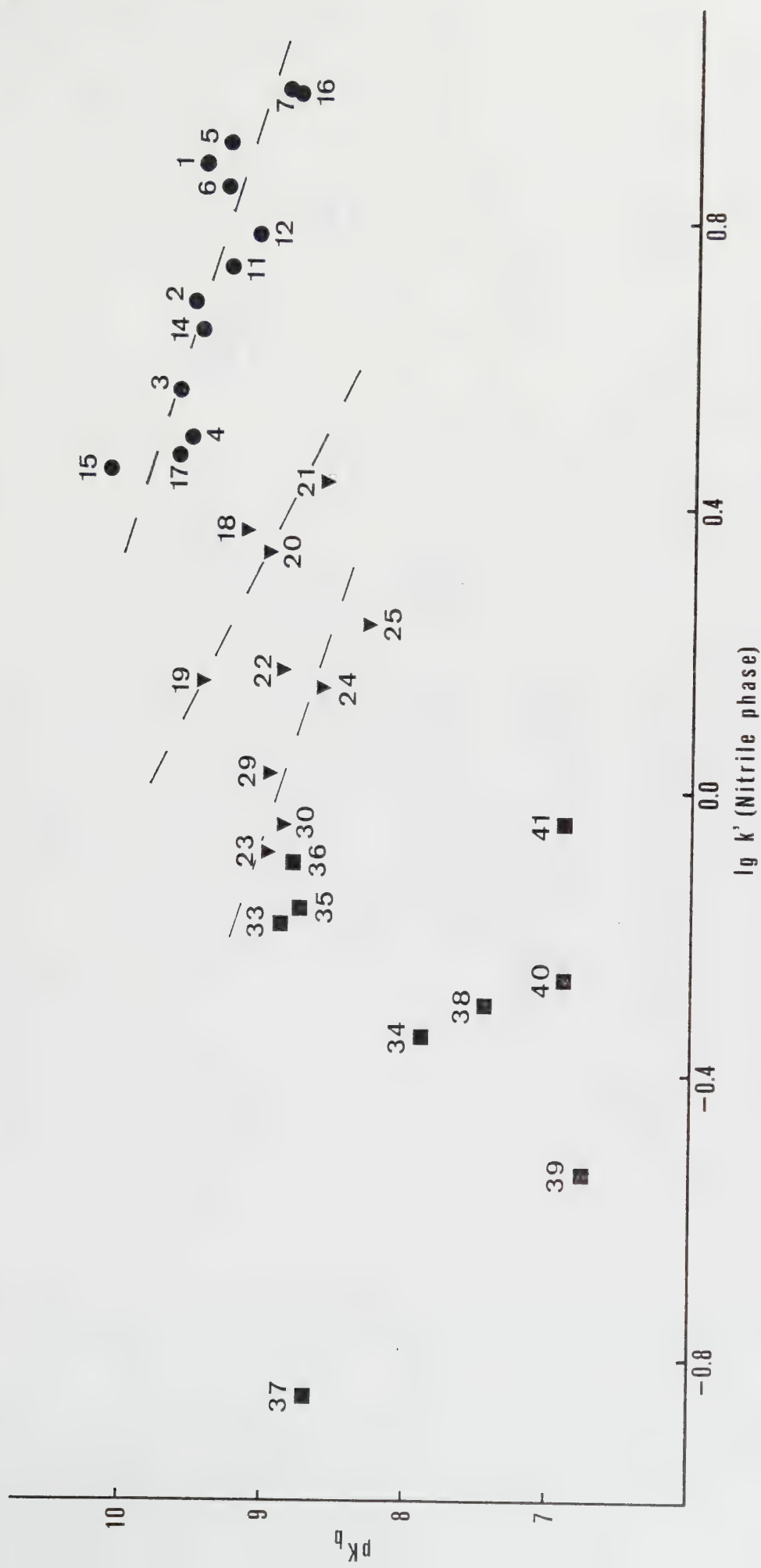


Fig. 2. Relationship between pK_b and $\lg k'$ of alkylanilines on the nitrile phase. Stationary phase, Nucleosil CN; mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml $\cdot$ h $^{-1}$. ● = Prim. anilines; ▼ = sec. anilines; ■ = tert. anilines. The numbers refer to Table I.

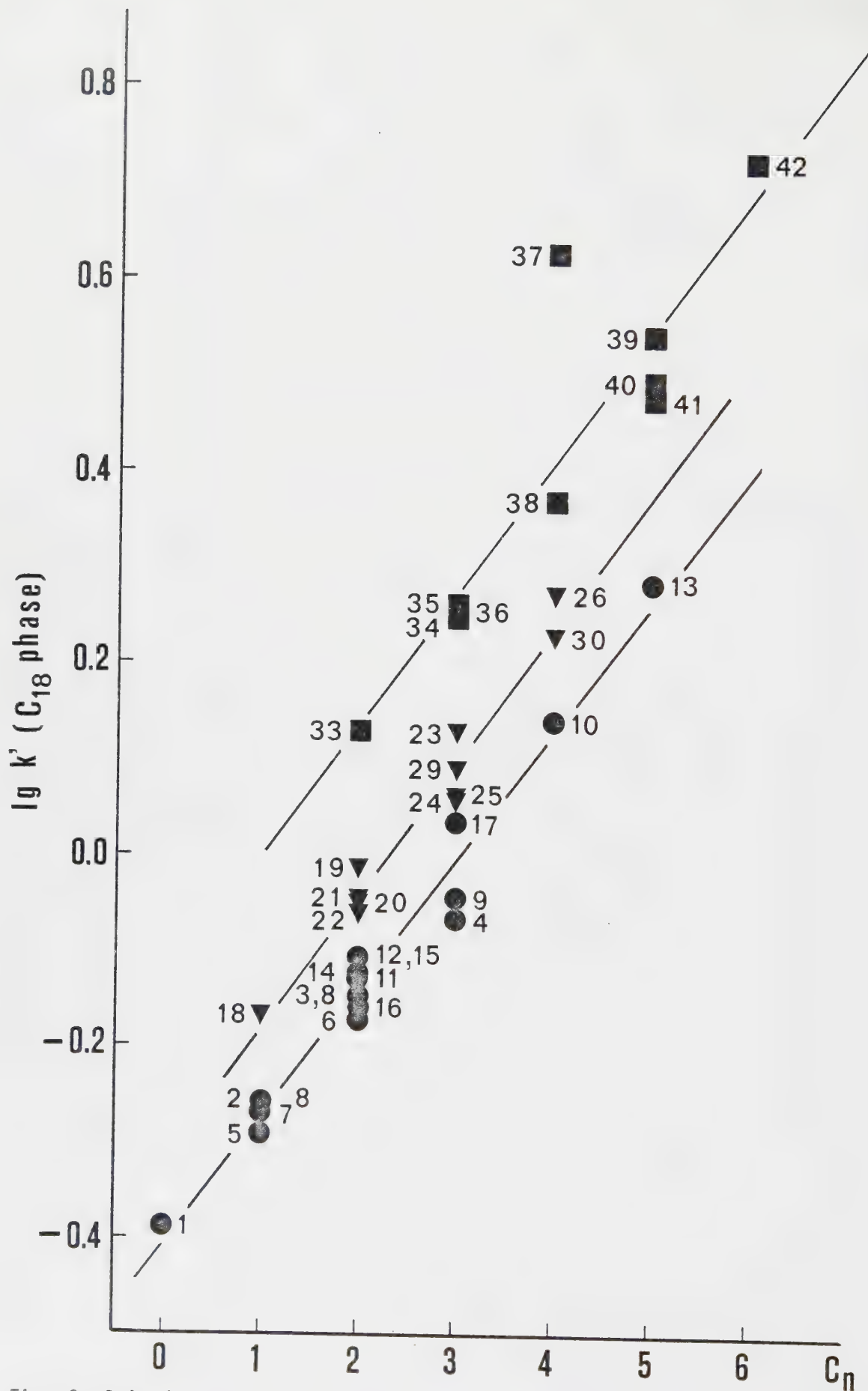


Fig. 3. Relationship between alkyl carbon number (C_n) and $\lg k'$ of alkyl-anilines on the C_{18} phase. Stationary phase, Nucleosil C_{18} ; mobile phase, methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml $\cdot$ h $^{-1}$. $\bullet$ = Prim. anilines; ∇ = sec. anilines; $\blacksquare$ = tert. anilines. The numbers refer to Table I.

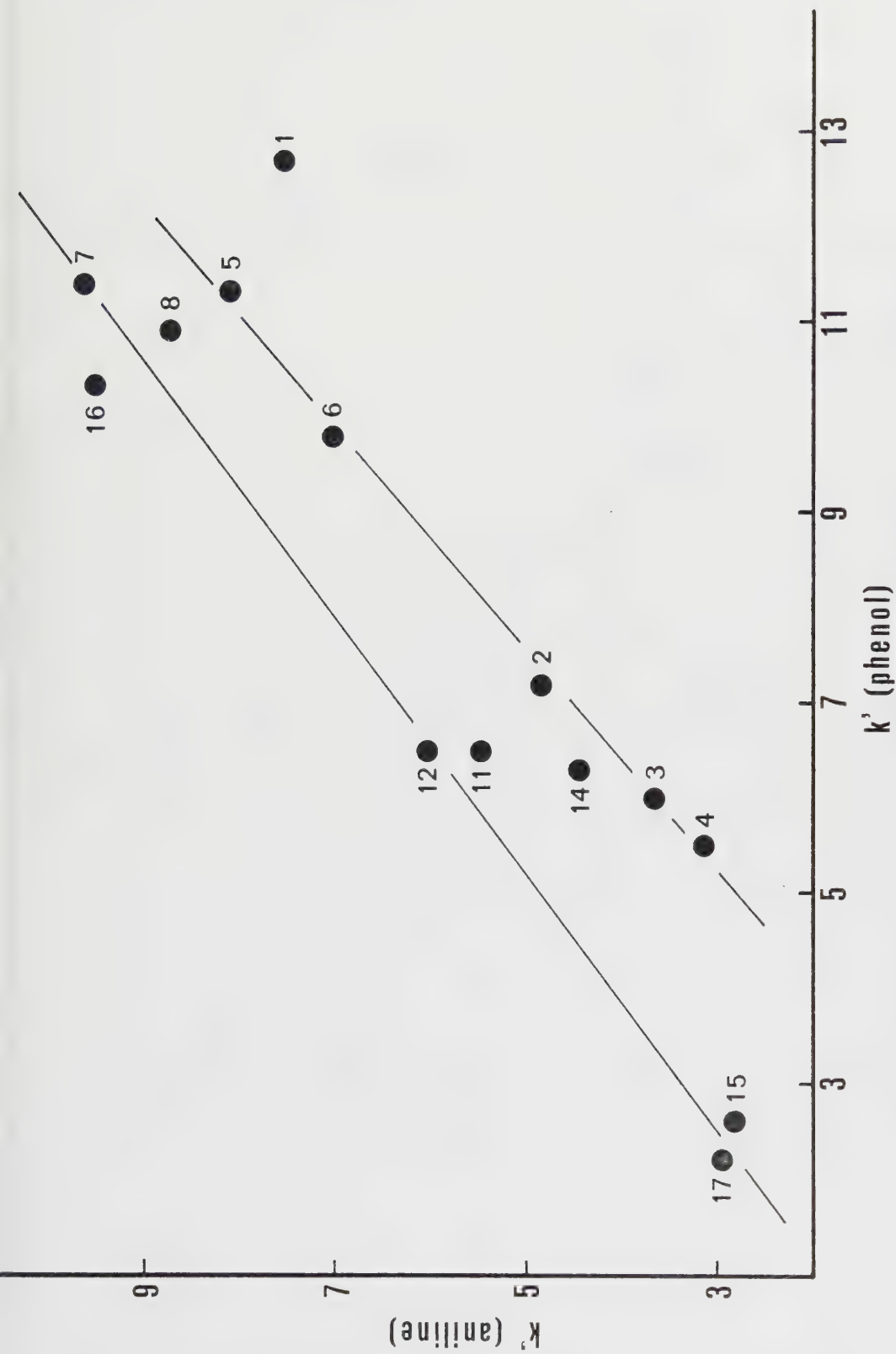


Fig. 4. Relationship between k' values of alkylanilines and k' values of corresponding alkylphenols on nitrile phases. Stationary phase anilines, Nucleosil CN; mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml $\cdot$ h $^{-1}$. Stationary phase phenols, Cyano Sil-X-1; mobile phase, 0.5% (v/v) 2-propanol in isooctane; 40 ml $\cdot$ h $^{-1}$. The numbers refer to Table I.

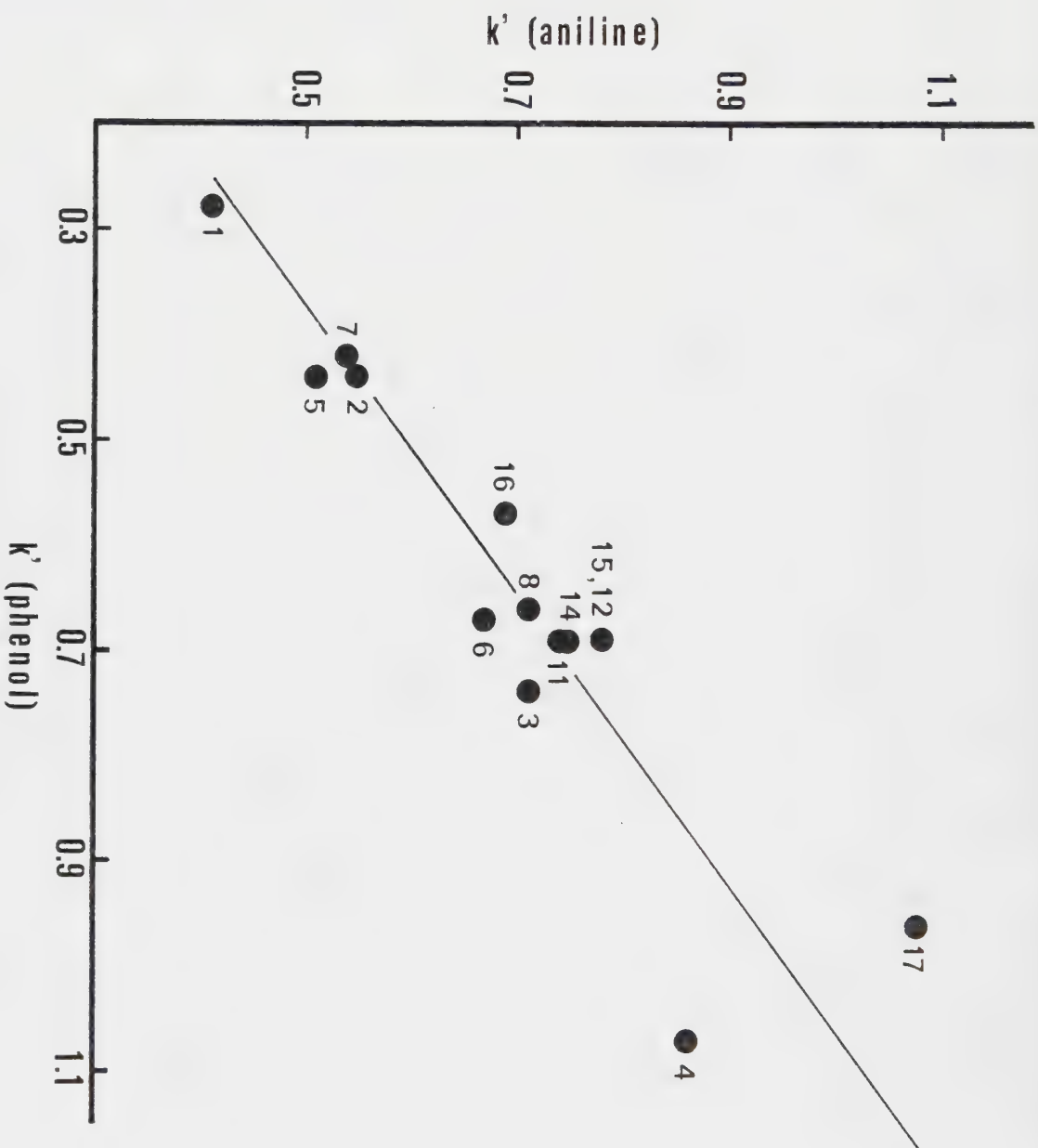


Fig. 5. Relationship between k' values of alkyl anilines and k' values of corresponding alkylphenols on C_{18} phases. Stationary phase anilines, Nucleosil C_{18} ; mobile phase, methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml $\cdot$ h $^{-1}$. Stationary phase phenols, μ Bondapak C_{18} ; mobile phase ethanol-water (60:40, v/v); 20 ml $\cdot$ h $^{-1}$. The numbers refer to Table I.

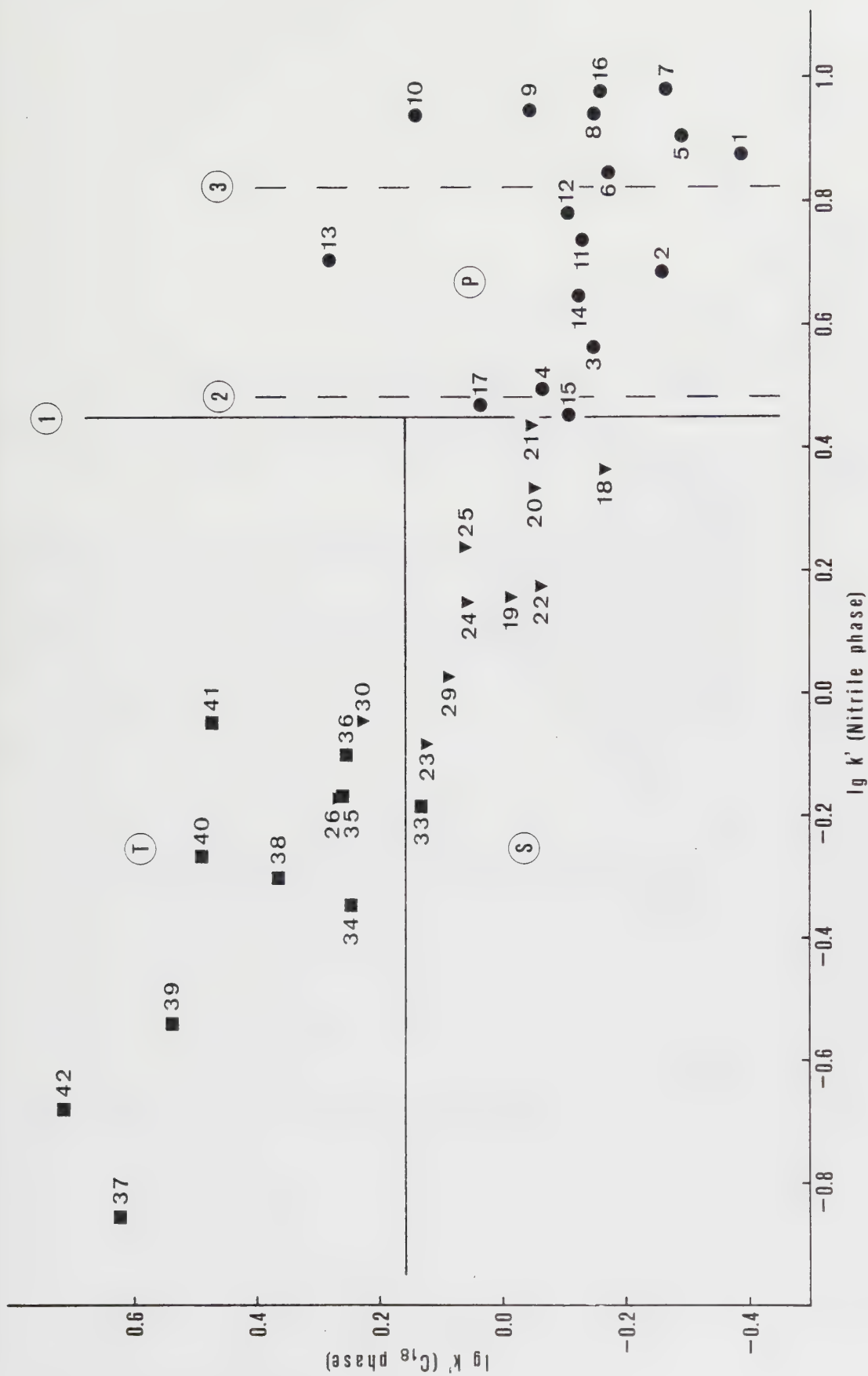


Fig. 6. Two-phase plot of $\lg k'$ of alkylanilines on reversed phase [Nucleosil C_{18} , methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml $\cdot$ h $^{-1}$] versus $\lg k'$ on straight phase (Nucleosil CN, 0.2%, v/v 2-propanol in isooctane; 45 ml $\cdot$ h $^{-1}$). ● and P = Prim. anilines; ▼ and S = sec. anilines; ■ and T = tert. anilines. The numbers refer to Table I.

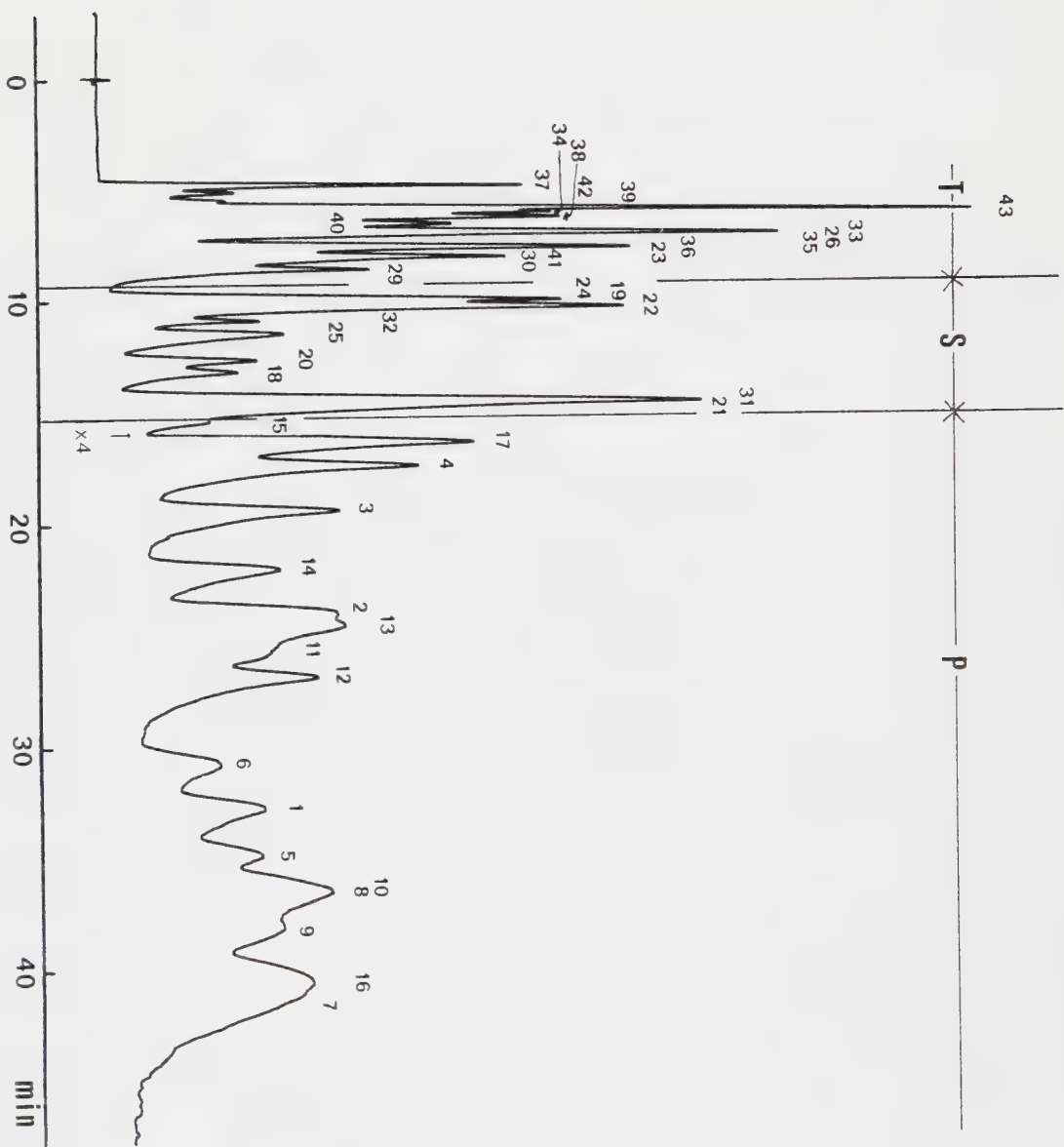


Fig. 7. Class separation of a mixture of prim., sec. and tert. alkyl anilines on the nitrile phase. Stationary phase, Nucleosil CN; mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml · h⁻¹. P = prim. anilines; S = sec. anilines; T = tert. anilines. The numbers refer to Table I.

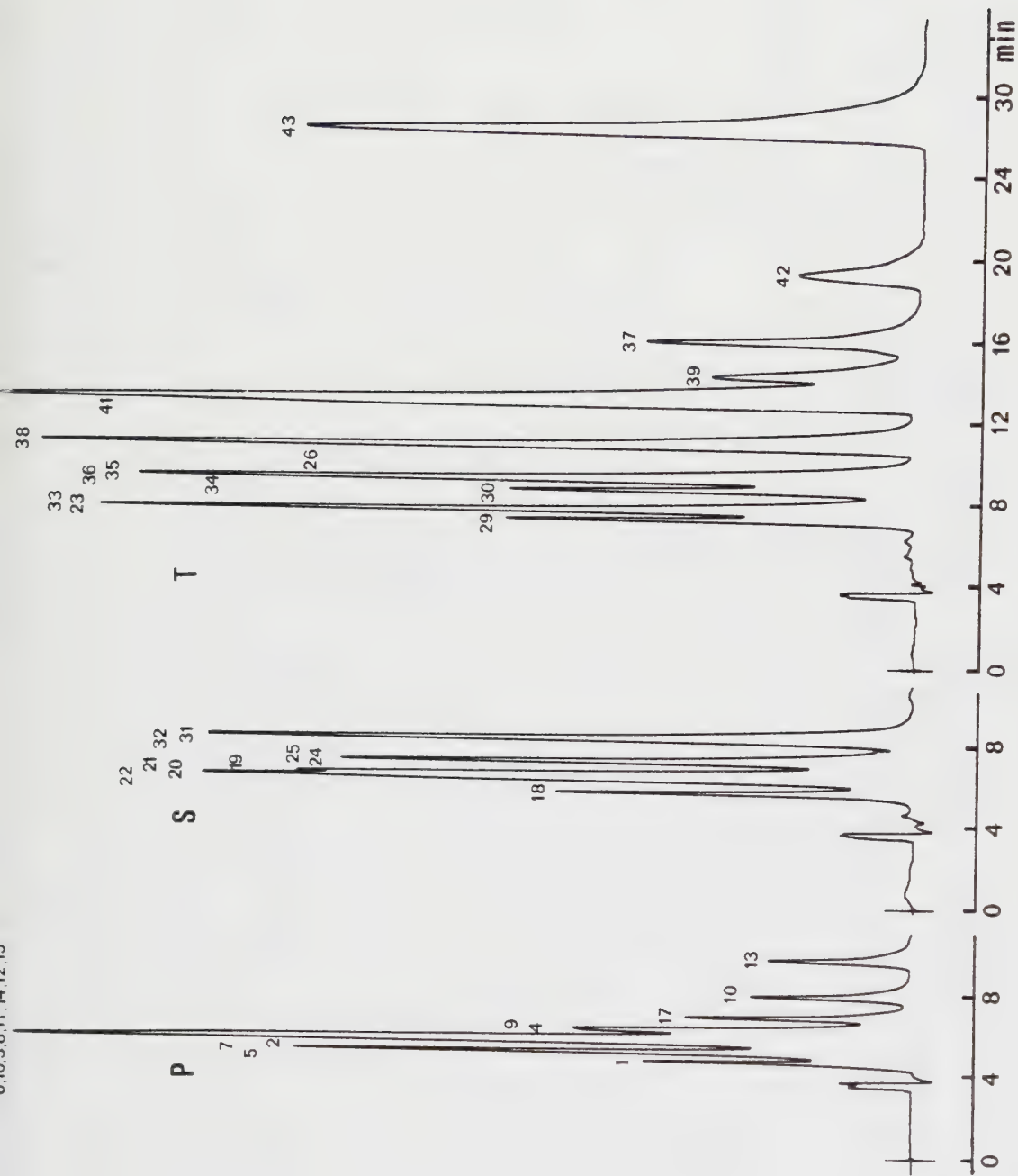


Fig. 8. Carbon number separation on the C_{18} phase of prim., sec. and tert. anilines, respectively, isolated from the run of the total mixture on the nitrile phase (Fig. 7). Stationary phase, Nucleosil C_{18} ; mobile phase, methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml $\cdot$ h $^{-1}$. P = prim. anilines; S = sec. anilines; T = tert. anilines. The numbers refer to Table I.

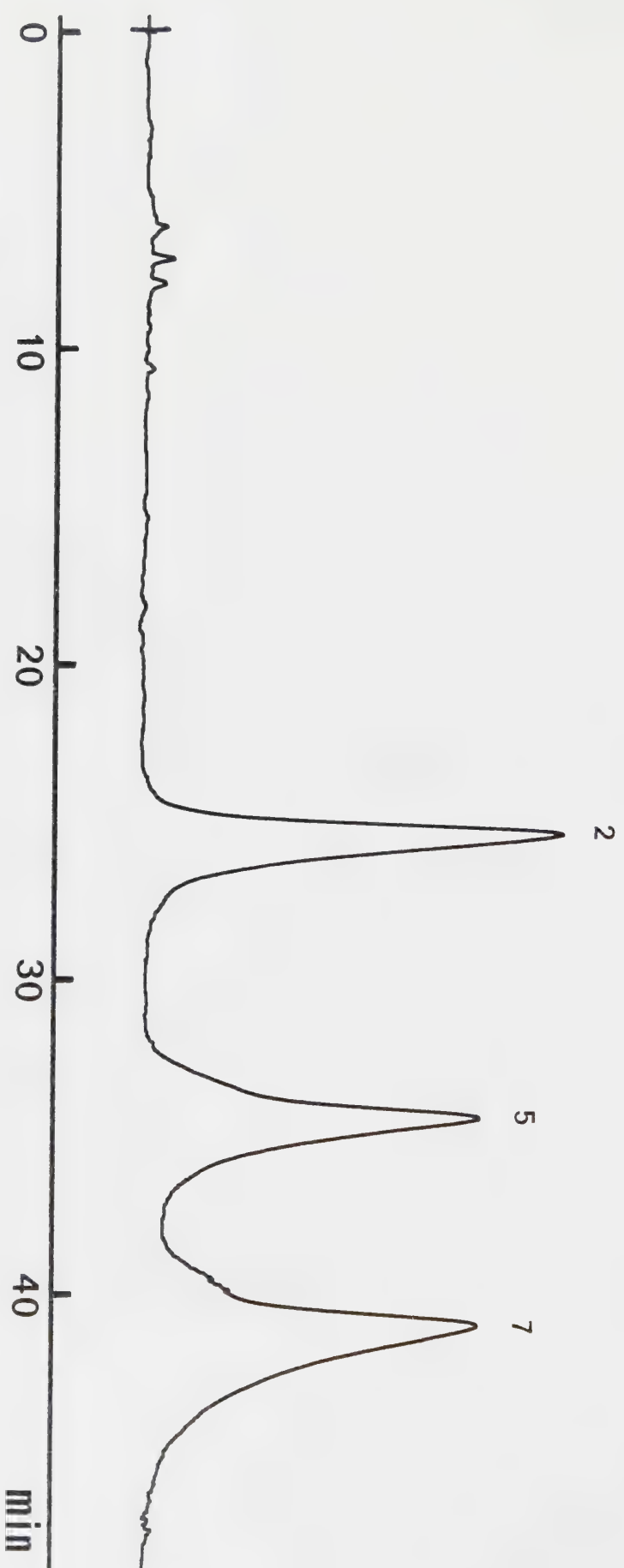


Fig. 9. Separation on the nitrile phase of prim. anilines, present in the peak with carbon number one, isolated from the carbon number run of prim. anilines (Fig. 8). Stationary phase, Nucleosil CN; mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml $\cdot$ h $^{-1}$. The numbers refer to Table I.

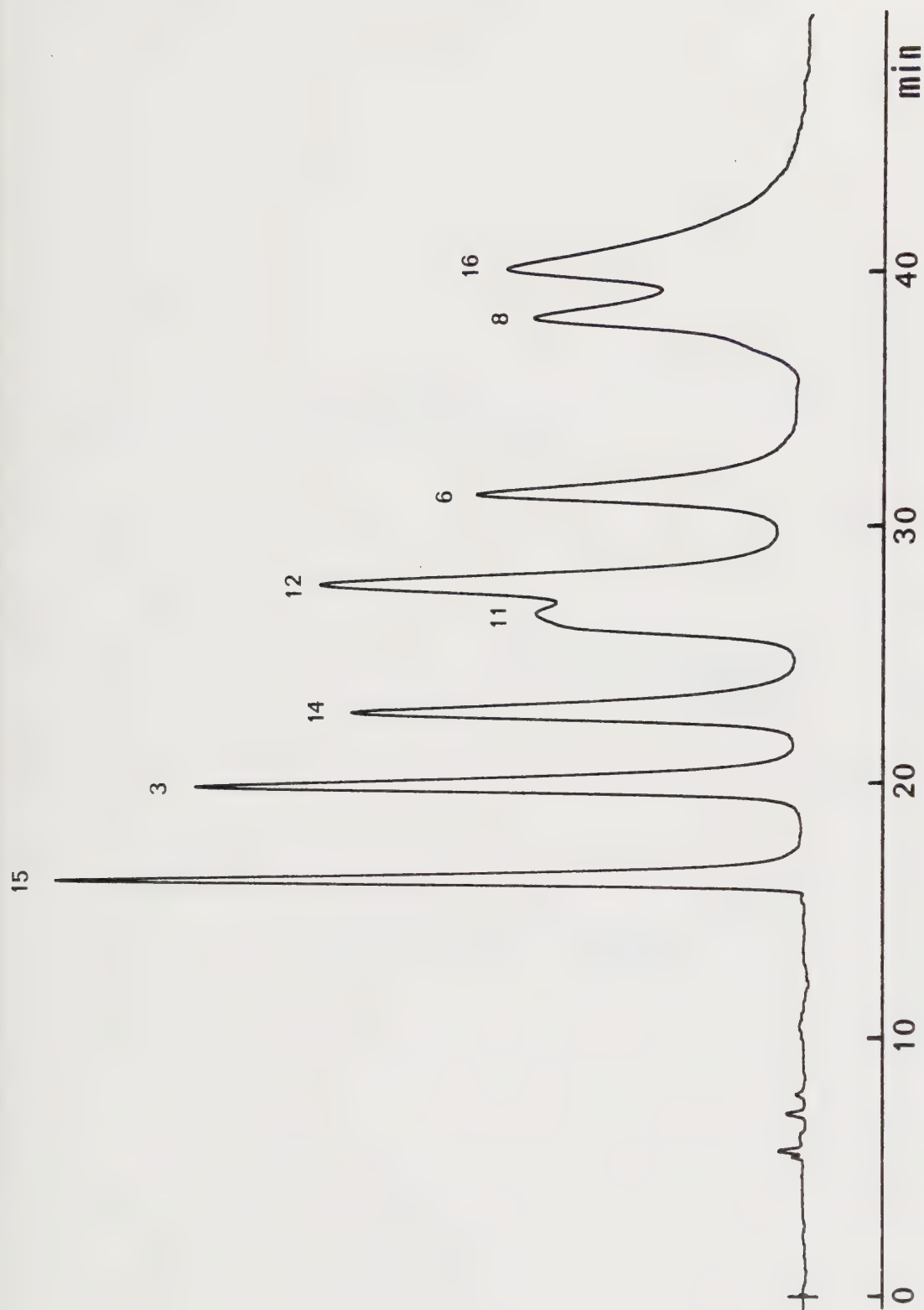


Fig. 10. Separation on the nitrile phase of prim. anilines, present in the peak with carbon number two, isolated from the carbon number run of prim. anilines (Fig. 8). Stationary phase, Nucleosil CN; mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml $\cdot$ h $^{-1}$. The numbers refer to Table I.

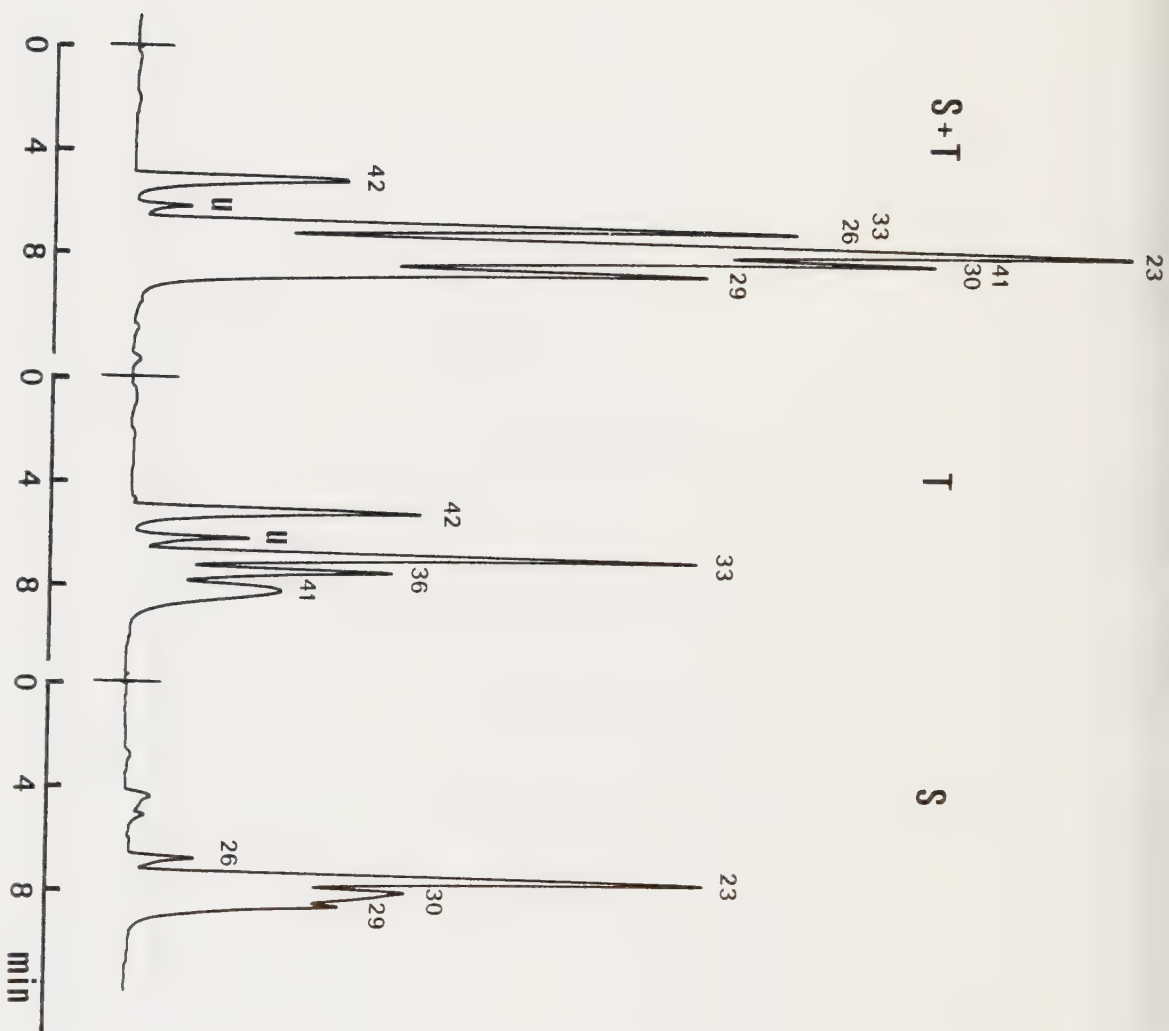


Fig. 11. Application of the acetylation procedure to a mixture of sec. (S) and tert. (T) anilines. (S+T) = chromatogram of mixtures on the nitrile phase. S and T = chromatograms of the isolated sec. and tert.

anilines, respectively, on the nitrile phase. Stationary phase, Nucleosil CN; mobile phase, 0.2% (v/v) 2-propanol in isooctane; 45 ml · h⁻¹. The numbers refer to Table I. u = unknown.

IV

LIQUID CHROMATOGRAPHY STUDY OF BROMINATED ANILINES AND INVESTIGATION
OF PRODUCT FORMATION IN THE BROMINATION REACTION

I. ANILINES WITHOUT RING SUBSTITUENTS OR WITH ALKYL GROUPS IN THE
ORTHO- AND PARA-POSITIONS

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SUMMARY

Straight- and reversed-phase liquid chromatography (LC) have been used to study product formation in the quantitative coulometric bromination of the headlined anilines. The coulometric method yields up to the end-point predictable, unambiguous products by exchange of hydrogen for bromine in free ortho- and para-positions. After the end-point, oxidation products may be formed from prim. anilines and N-alkyl groups split off from sec. and tert. anilines.

A detailed study of retention behaviour of some 70 bromoanilines in straight- and reversed-phase LC has been made. It turned out that an increase in retention generally took place on the introduction of bromine into the aniline nucleus, but for the formation of o-bromoanilines in straight-phase LC, where retention decreases. Retention behaviour of bromoanilines in straight-phase LC is discussed in terms of base strength, steric hindrance around the nitrogen atom and solvent effect. A semi-linear relationship was found to exist between capacity factors of brominated and non-brominated anilines in both of the LC systems.

INTRODUCTION

In a previous paper¹ it was shown that alkylanilines can be quantitatively brominated with a coulometric technique, based on reaction with anodically generated bromine. The advantage of that method compared with other methods, based mainly on volumetric bromination², is that the reaction can be controlled by means of the titration medium and by using an optimal generating current². The method can therefore be applied to a large number of anilines, even those which are usually sensitive to side-reactions like oxidation.

The aim of this investigation was to study product formation by liquid chromatography (LC) at various stages during the coulometric bromination and at the same time to examine the retention behaviour of brominated alkylanilines in different LC systems. In this work the choice of anilines has been restricted to compounds which either have no ring substituents or contain alkyl groups in the ortho and/or para positions, i.e. 2- and 4-monoalkylanilines and 2,4- and 2,6-dialkylanilines. Prim., sec. as well as tert. anilines are included. In a forthcoming paper, meta-alkyl-substituted alkylanilines will be treated.

EXPERIMENTAL

Apparatus

Coulometric titration. The apparatus and procedure for the coulometric bromination have been described in detail elsewhere^{1,3}.

Liquid chromatography. The LC pump used was a Varian Model 4100 (Varian, Palo Alto, Calif., U.S.A.) and the detector was a Laboratory Data Control Model 1285 UV monitor (Laboratory Data Control, Riviera Beach, Fla., U.S.A.) used at 280 nm. Sample application was accomplished by a valve injector (Rheodyne, Berkeley, Calif., U.S.A.) with a 20 μ l loop.

Columns. The bonded phase packing materials were the commercially available Nucleosil C₁₈, 5 μ m and Nucleosil CN, 5 μ m (Machery-Nagel & Co., Düren, G.F.R.). They were packed in accordance with the upward-slurry packing technique^{4,5}. All columns consisted of precision-bore stainless steel tubing (4.4 mm I.D. x 200 mm). The columns were used at room temperature.

For accurate work it is necessary to reactivate the columns, especially the nitrile column, regularly. This was made according to recommended procedures. The stability of the columns was tested daily using a mixture of phenol, 2,6-dimethylphenol and 4-tert.butylphenol for the reversed-phases and a mixture of diphenylamine, triphenylamine and N-methyl-2-methylaniline for the nitrile phase.

Chemicals

Isooctane (certified A.C.S. grade, Fisher Scientific, Fairlawn, N.J., U.S.A.), methanol (analytical-reagent grade, May & Baker, Dagenham, Great Britain), 2-propanol (pro analysi grade, E. Merck, Darmstadt, G.F.R.), sodium dihydrogen phosphate, NaH_2PO_4 , $2\text{H}_2\text{O}$ (99%, BDH Chemicals, Poole, Great Britain), disodium hydrogen phosphate, Na_2HPO_4 , $2\text{H}_2\text{O}$ (according to Sörensen, E. Merck) and orthophosphoric acid (pro analysi grade, E. Merck) were used for preparing the LC eluents.

Acetic acid (pro analysi grade, E. Merck) and sodium bromide (99%, BDH) were used for the preparation of the titration media.

The anilines were of the best grade commercially available. Some of them were further purified by distillation or recrystallization. N,N-Dimethyl-2,6-dimethylaniline, N,N-diethyl-2-ethylaniline and N-ethyl-2-ethylaniline were prepared at this laboratory.

Mobile phases

For reversed-phase LC on the C_{18} phase, methanol-aqueous buffer was used and for straight-phase LC on the nitrile phase isooctane, containing 0.2% (v/v) 2-propanol, was applied.

Methanol-aqueous buffer (70:30, v/v, pH = 7.0). Fifteen ml of 0.025 mole l^{-1} Na_2HPO_4 + 250 ml of 0.025 mole l^{-1} NaH_2PO_4 + 618.33 ml of methanol; pH was adjusted to 7.0 with small amounts of orthophosphoric acid and dilute sodium hydroxide (1 mole l^{-1}).

Methanol-aqueous buffer (80:20, v/v, pH = 7.0). Fifteen ml of 0.05 mole l^{-1} Na_2HPO_4 + 250 ml of 0.05 mole l^{-1} NaH_2PO_4 + 1060 ml of methanol; pH was adjusted as above.

Procedure

Coulometric titration. Anilines, ca. 20 $\mu\text{equiv.}$ in 20 ml titration medium were coulometrically brominated as described by Truedsson and Smith¹. All titrations were performed in medium III-1 (acetic acid - water

(60:40, v/v), bromide concentration 0.1 mole l^{-1} .

Chromatography. For the reversed-phase studies $20 \mu\text{l}$ aliquotes containing ca. 5 nmole were removed at different stages of the titration directly from the titration vessel by means of a micro-syringe, after stopping the generating current. The samples were then injected directly on to the column. In the case of the nitrile phase studies, an extraction procedure was included in order to remove the acetic acid and water.

Extraction procedure. An aliquote (4 ml) of the titration mixture was transferred to a 50 ml separating funnel and 5 ml of water and 5 ml of isooctane were added. After shaking, sodium hydroxide (10 mole l^{-1} , $4\text{--}5 \text{ ml}$) was added in order to make the water phase alkaline and the mixture was shaken again. The isooctane phase was removed and dried over sodium sulphate and the solvent partly evaporated with a gentle flow of nitrogen to a volume of $1\text{--}2 \text{ ml}$. From this solution, $20 \mu\text{l}$ were injected on to the nitrile phase column.

Capacity factor, k' . The capacity factor given is a mean value from at least three injections with a relative standard deviation of about 3%. Retention times of unretained solutes were determined by injecting n-hexane on the nitrile phase and aqueous sodium nitrate solution (0.05%) on the reversed-phases. The capacity factor k' was calculated from the following formula

$$k' = \frac{t_R - t_0}{t_0} \quad (1)$$

where t_R = retention time of sample,

t_0 = retention time of unretained solute.

Separation factor, α . The separation factor for a substance pair A and B is obtained by division of their capacity factors

$$\alpha_{A,B} = \frac{k'_A}{k'_B} \quad (2)$$

RESULTS AND DISCUSSION

Choice of liquid chromatographic systems

In a previous investigation⁶ it was shown that valuable information about the structure of different kinds of anilines can be gained

by a combination of straight- and reversed-phase LC.

For the study of brominated anilines in this work the same kinds of chromatographic systems were chosen, viz. one straight-phase system on nitrile phase with isooctane, containing 0.2% (v/v) 2-propanol, as eluent and one reversed-phase octadecylsilane (C_{18}) system with methanol-aqueous buffer as eluent. Two eluents were used, one with methanol-aqueous buffer (70:30, v/v) and one with the proportions 80:20 (v/v). In both cases were the eluents buffered at pH 7.0 in order to improve peak symmetry and reduce the tendency to formation of double peaks.

It appeared, on varying the methanol content in the eluent, that the retention of brominated anilines on the C_{18} phase decreased when the percentage of methanol increased. In order to obtain appropriate elution times and good resolution, the composition methanol-aqueous buffer (70:30, v/v) was chosen for brominated prim. anilines and the composition 80:20 (v/v) for brominated sec. and tert. anilines. However, some prim. anilines were for comparison also run with the latter eluent. On the nitrile phase, retention of brominated anilines is very much dependent on the content of 2-propanol in the isooctane eluent. On that account, the eluent must be carefully prepared and it is also essential to use dry solvents.

Introduction of sample. On the reversed-phase column, direct injection of aliquotes taken from the titration vessel was carried out without any disturbances of the chromatogram or impairment in column performance. On the straight-phase system, however, an extraction procedure had to be put in before injection, in order to remove acetic acid and water as previously described.

Product formation in coulometric bromination

In the coulometric bromination method for the titration of anilines described by the present authors¹, the reaction is carried out in a water-acetic acid medium and the reactivity is controlled by varying the water content and the bromide ion concentration and by the addition of pyridine. The reaction is believed to involve substitution with bromine at free ortho- and para-positions. For anilines with several such positions, the titration can be carried out to either the fully brominated stage or to a stage corresponding to the introduction of a smaller number of bromine atoms than the number of available free ortho- and

para-positions. For a certain aniline, the outcome of a titration is dependent both on the structure and on the bromination-promoting properties of the titration medium.

In the present investigation, the aim was to study product formation during titration in the pyridine-free medium III-1, containing acetic acid and water in the proportions 60:40 (v/v) and with a bromide concentration of 0.1 mole l^{-1} . For this purpose samples were removed from the titration vessel at various stages of the titration and injected on to the LC columns either directly (C_{18} phase) or after extraction (nitrile phase). Fig. 1, which gives a typical titration curve, illustrates the procedure. Samples were removed at points indicated by A, B, C and D, and analyzed. The resulting chromatograms obtained for 2-methylaniline can be seen in Fig. 2. At point A, half-way to the end-point, the main bromination products formed are 4-bromo-2-methylaniline (c) and 4,6-dibromo-2-methylaniline (e), while very little of the 6-bromoderivative (d) appears. At the end-point (B) only the dibrominated product (e) is present. Analogous results were obtained for other 2-alkylanilines when titrated in medium III-1.

The results of the monobromination of different kinds of anilines up to point A on the titration curve can be gathered from Table I, which gives the k' values on the C_{18} phase of the various bromoanilines formed. As can be seen, all tested prim. anilines with a free ortho-position, yield an ortho-bromoderivative, while sec. and tert. anilines only furnish this derivative when the para-position is occupied by an alkyl group. The increased steric hindrance at the ortho-positions of sec. and tert. anilines obviously precludes the introduction of a bromine atom, unless the para-position is occupied.

Diphenylamine constitutes an exception from other sec. anilines in that an ortho-bromoderivative is formed on bromination in medium III-1. Samples taken during the bromination show, that the order of formation of bromoderivatives is 2- and 4-monobromo-, 2,4'- and 4,4'-dibromo-, 2,4,4'-tribromo- and 2,2',4,4'-tetrabromodiphenylamine.

Triphenylamine, however, does not yield any ortho-bromoderivative. In this case the order of formation of bromoderivatives is 4-monobromo-, 4,4'-dibromo- and 4,4',4''-tribromotriphenylamine.

Effect of over-bromination. All evidence points to the fact that coulometric bromination of anilines up to the end-point in the proper

titration medium proceeds with the formation of bromoanilines due to exchange of hydrogen for bromine at free ortho- and para-positions. However, it is well known that, in the analysis of anilines by volumetric bromination, some compounds are able to consume more bromine than the stoichiometric amount corresponding to the free ortho- and para-positions. Examples of side-reactions that have been suggested or proved to take place are replacement of ortho- and para-situated groups with bromine and various oxidation reactions.⁷⁻¹⁰

The reason for the good quantitative results obtained on coulometric bromination of anilines in comparison with results from volumetric bromination, is undoubtedly the careful choice of proper titration media and the fact that an excess of bromine is never allowed to build up before the end-point. Nevertheless, it was considered to be of interest also to examine the behaviour of the anilines in question after the end-point. For this purpose an excess of bromine was generated which depended on the kind of aniline. Thus, for prim. anilines, where all vacant ortho- and para-positions are substituted with bromine at the end-point, an excess of about 50% of bromine was generated (point C in Fig. 1). Sec. and tert. anilines, which are not full-brominated in medium III-1, were exposed to an excess of bromine corresponding to full bromination (point D in Fig. 1).

Among prim. anilines, ortho-alkyl-substituted compounds seem to be particularly prone to react further on over-bromination. Thus, 2,6-dimethylaniline formed an over-bromination product which was investigated by means of gas chromatography-mass spectrometry and nuclear magnetic resonance and shown to be 4-amino-4'-bromo-3,5,2',6'-tetramethyldiphenylamine (b in Fig. 3). It is accordingly an oxidation product from 4-bromo-2,6-dimethylaniline, which latter compound is present at the end-point. This oxidation product is formed rather soon after the end-point, in fact, a small amount is present even before. We have also found it to arise on volumetric over-bromination and similar structures have been isolated on anodic oxidation of p-chloroaniline¹¹.

Further generation of bromine in the reaction mixture of 2,6-dimethylaniline yielded another product which, on the nitrile phase, was eluted nearer the front than the diphenylamine above (a in Fig. 3). It was tentatively identified as 4,4'-dibromo-2,6,2',6'-tetramethylhydrazobenzene, a type of compound which has been reported to be formed

on anodic oxidation of anilines¹¹. As shown by the small peaks (u in Fig. 3), other over-bromination products are formed, the nature of which, however, is as yet unknown.

On over-bromination of 2-methylaniline, some early peaks of unknown origin appear at separation on the C₁₈ phase (u in Fig. 2C). They are formed from 2-methyl-4,6-dibromoaniline (e) and spectral evidence indicates that their structure is quinoidic. These peaks are late on the nitrile phase in contrast to the substituted diphenylamine and hydrazobenzene, previously discussed in connection with over-bromination of 2,6-dimethylaniline (a and b in Fig. 3).

Para-alkyl-substituted prim. anilines show a far better stability against excess of bromine than ortho-substituted. The 4-alkylanilines, for example, gave essentially the same chromatogram at 50% over-bromination as at the end-point.

Sec. and tert. anilines of most of the kinds studied in this work are not full-brominated at the end-point in medium III-1, but still contain vacant ortho-positions. The result of over-bromination is, that these free positions are substituted with bromine, producing the full-brominated aniline. However, this reaction cannot be utilized for quantitative purposes. The full-brominated sec. and tert. anilines are very sensitive to further excess of bromine and react with loss of N-alkyl groups to the corresponding prim. and sec. anilines (Fig. 4). An exception from this reaction order was constituted by N,N-diethylaniline. This compound is present as the 4-bromoderivative at the end-point and, on additional generation of bromine, one of the N-ethyl groups is split off, before further bromination takes place.

Retention behaviour of brominated anilines

Reversed-phase chromatography. The retention of solutes in reversed-phase chromatography is determined primarily by dispersion forces between the solute and the stationary phase and by the solubility in the mobile phase.¹²⁻¹⁴ The size and shape of the molecule is of importance for the magnitude of the dispersion forces. Thus, it has been shown that the retention of prim., sec. and tert. anilines in reversed-phase chromatography is mainly determined by the total alkyl carbon number, representing the sum of the nuclear and N-substituted alkyl groups⁶.

The introduction of bromine into the aniline nucleus gives a considerable increase in retention in reversed-phase chromatography (Table I and Figs. 5(a) and 6(a)). The great molar volume of the bromine atom is considered to be of special importance for this change in retention, decreasing the solubility of the bromoanilines in the eluent¹⁵.

There exists a semi-linear relationship between k' values of the original anilines on one hand, and k' values of the corresponding mono-, di- and tribrominated derivatives, respectively, on the other hand, as demonstrated by Fig. 7.

(a) Prim. anilines. When bromine is substituted into aniline itself or into a 2- or 4-alkylaniline, retention on the C_{18} phase increases with the number of bromine atoms entering. This is evident from the values of the separation factors, α , given in Table I. Among monobrominated anilines, the para-bromo-isomer is eluted before the corresponding ortho-bromo-isomer. Since e.g. o-bromoaniline is a weaker base than p-bromoaniline, it seems that the elution order is governed by the base strength, the least basic amine being eluted last. It is of interest to note that the same rule applies to o- and p-bromophenol on the C_{18} phase¹⁶. In this case p-bromophenol, which is the weakest acid, is eluted last. These facts are in accordance with the hydrophobic theory put forward by Horváth et al.¹⁷ for the interaction between solutes and hydrocarbonaceous bonded stationary phases, using mixtures of water and organic solvents as mobile phases.

The retention effect of introducing bromine into a prim. aniline is demonstrated in Figs. 8a and b, where the separation factors, α , for the monobrominated aniline and the aniline, respective the di- and mono-brominated anilines are plotted against carbon number. As can be seen, the increase in retention on mono-ortho-bromination is greater than on mono-para-bromination (Fig. 8a).

On further bromination of the originally formed o-bromoanilines, either o,p- or o,o-dibromoanilines are formed. As shown by Fig. 8b, the separation factors are slightly higher for the o,o-dibromoanilines, which is in accordance with the results from the monobromination. Dibromination of the p-bromoanilines yields only one product viz. p,o-dibromoanilines. In this case the separation factor for the di- and monobromination products is distinctly higher than for the above-mentioned series of dibrominated anilines.

The difference between, and relative constancy of, the separation factors for compounds formed on mono- and dibromination of prim. anilines, makes it possible to predict the order of elution of bromination products formed on coulometric bromination of this kind of anilines, thereby facilitating the interpretation of reversed-phase chromatograms. The linear relationship between k' values of brominated and non-brominated prim. anilines is also of value for this purpose.

The elution order on the C_{18} phase of aniline and some prim. methyl-anilines and their bromination products is demonstrated in Fig. 5a, which also gives a further illustration of the retention rules previously discussed.

(b) Sec. and tert. anilines. As for prim. anilines, there exists a semi-linear relationship between k' values of non-brominated anilines and k' values of mono-, di- and tri-brominated analogues, respectively (Fig. 7). However, it is obvious that certain compounds show a considerable deviation from a linear relationship.

The plot of separation factors against carbon numbers in Figs. 9a and b gives information about the retention change caused by the introduction of one, two and three bromine atoms, respectively, into a sec. aniline. As previously established for prim. anilines, the increase in retention on mono-ortho-bromination is greater than on mono-para-bromination (Fig. 9a). On further bromination of o-bromoanilines, o,o-dibromoanilines are formed. The increase in retention on this bromination is smaller than when a p-bromoaniline is brominated to a p,o-dibromoaniline, just as for the corresponding prim. anilines. For the sec. p,o-dibromoanilines two different series can be discerned, viz. compounds with or without an ortho-situated alkyl group. The separation factors for the former series are lower and do not change with the carbon number. For the latter series the separation factor increases linearly with the carbon number, i.e. with the size of the N-alkyl group, when the first ortho-situated bromine atom is introduced, but remain constant when, on tribromination, the second ortho-standing bromine atom is substituted (Fig. 9b).

For brominated tert. anilines the retention pattern on the C_{18} phase is somewhat different from that of prim. and sec. anilines. Thus, for the latter two groups the greatest change in retention, on monobromination, occurred on formation of o-bromoanilines from anilines, and the smallest change, on dibromination, occurred on formation of o,o-dibromoanilines from

o-bromoanilines. For tert. anilines the reverse is true as shown by Figs. 10a and b. There is also a reversal of the separation factors for the two series formed on ortho-bromination of p-bromoanilines.

It has been stated by Locke¹⁴ that for compounds of similar type, selectivity in reversed-phase chromatography is primarily determined by the eluent and that elution order is in the inverse order of solute solubilities in the moving phase. However, it is uncertain to what extent the actual anilines should be regarded as being of "similar type" and it is more likely that the disparity in screening and steric hindrance between different ortho-brominated anilines affects the dispersion forces acting upon the molecule as well as its hydrophobicity.

The elution order on the C₁₈ phase of some sec. and tert. anilines and their bromination products is demonstrated in Fig. 6a, which also gives a further illustration of the retention rules discussed above.

Straight-phase chromatography. In a previous work,⁶ it was shown that the elution order on the nitrile phase of different kinds of anilines is tert., sec. and prim. anilines and that the retention within each group is mainly determined by the number and size of ortho-situated alkyl groups and by alkyl groups substituted at the nitrogen atom.

The introduction of bromine into the aniline nucleus changes the elution order, and it is no longer possible to decide straight off, on the basis of k' values, if a brominated aniline is primary, secondary or tertiary. This fact is evident from the k' values presented in Table II. However, it can be seen that within each subgroup of brominated anilines, i.e. mono-ortho-bromo-, mono-para-bromo- etc., the k' values are to a great extent dissimilar and often allow the distinction between prim., sec. and tert. bromoanilines of different kinds (Table III).

The most striking nitrile phase retention effect of the introduction of bromine into an aniline is the great decrease in retention (with some exceptions) on ortho-bromination. This effect is primarily thought to be caused by steric hindrance due to the bromine atom, which restricts the interaction between the amino group and the nitrile group in the bonded phase. The substitution of bromine into the para-position of an aniline leads to either an increase in retention or to a small decrease, depending on the kind of aniline. This fact is further illustrated in Figs. 5b and 6b, which give the elution order on the nitrile phase of aniline and some methyl-anilines and their bromination products.

The semi-linear relationship between k' values of brominated and original anilines, previously established for the C_{18} phase, is also valid for the nitrile phase (Fig. 11). However, because of the increased selectivity of the nitrile phase, o-bromo- and p-bromo-anilines fall on different lines and the same is true for o,o-dibromo- and o,p-dibromoanilines. The diagram in Fig. 11 is thus considerably more structurally informative than the corresponding diagram for the C_{18} phase in Fig. 7.

(a) Prim. anilines. In Figs. 12a and b the separation factors for the brominated prim. anilines on the nitrile phase have been plotted against carbon numbers. The great difference in retention behaviour on ortho- and para-bromination, respectively, is clearly demonstrated.

(b) Sec. anilines. Mono-ortho- and mono-para-bromination of sec. anilines give rise to a similar separation factor picture as for prim. anilines (Fig. 13a). However, on introduction of a second and a third bromine atom the picture changes (Fig. 13b). While the lines for the conversion of o-bromo- to o,o-dibromo- and p-bromo- to p,o-dibromoanilines coincide for prim. anilines, they are well separated for sec. anilines, and the retention change is far less for the former series. Hence, when 2-bromo-4-methyl-N-ethylaniline (No. 22) is brominated to 2,6-dibromo-4-methyl-N-ethylaniline the decrease in retention is very small.

The same tendency to a diminished change in retention on ortho-bromination of a sec. o-bromoaniline is shown on ortho-bromination of sec. o,p-dibromoanilines. Thus, when a third bromine atom is introduced into the ortho-position of these compounds the decrease in retention is considerably less than when 2,4-dibromoaniline is brominated to 2,4,6-tribromoaniline (Table II).

(c) Tert. anilines. Most notable on bromination of tert. anilines, is the behaviour on mono-para-bromination. While, on mono-para-bromination of prim. and sec. anilines, p-bromoanilines with a greater retention than the original aniline resulted, mono-para-bromination of tert. anilines produced p-bromoanilines which travelled either more slowly or more rapidly than the original aniline (Fig. 14a). The reason for this is discussed below.

Causes of retention change on bromination. In a previous study on retention behaviour of alkylanilines in straight-phase chromatography it was shown that retention was mainly governed by base strength and by the substitution pattern around the nitrogen atom⁶. This is partly true also for bromoanilines. Thus, the decrease in retention following ortho-bromination is considered to be due to a decrease in base strength and to increased steric hindrance to interaction between the amino group and the bonded phase nitrile group.

The fact that para-bromination generally causes an increase in retention cannot be explained in this way. Since the environment of the nitrogen atom is not changed on para-bromination and the base strength decreases, one would rather expect the retention to decrease on para-bromination. There is obviously a third factor which influences retention of p-bromoanilines. It is suggested that this factor is a decreased solubility of the p-bromoaniline in the mobile phase in comparison with the original aniline, causing it to travel more slowly than this compound.

Among para-bromo-substituted tert. anilines some compounds were eluted before the original aniline (Fig. 14a). This deviation from the general retention rule for p-bromoaniline is most likely a result of the interplay between the change in base strength and the change in solubility on bromination. In this case the decrease in solubility is not great enough to match the decrease in base strength, which causes the p-bromoaniline to travel more rapidly than the original aniline.

Although the base strength of the aniline, and especially steric effects, were considered mainly to govern retention change on ortho-bromination, solvent effects cannot wholly be left out of consideration. Thus, the different retention behaviour of certain sec. anilines on ortho-bromination compared to corresponding prim. and tert. anilines (Fig. 13b) may well be caused by solubility differences.

CONCLUSIONS

For the investigated anilines the coulometric bromination technique described by Truedsson and Smith¹ yields predictable, unambiguous products when the titration is continued to the end-point. In the reaction, hydrogen is exchanged for bromine at free ortho- and para-positions, with the formation of o- and p-bromoanilines.

Over-bromination of prim. anilines leads to oxidation products, especially for ortho-alkyl-substituted compounds, whereas para-alkyl-substituted prim. anilines show a far better stability against excess of bromine. The sec. and tert. anilines studied in this work are generally not fully brominated at the end-point, but still contain vacant ortho-positions. On over-bromination, these free positions are substituted with bromine and, on further bromination, loss of N-alkyl groups occurs with the formation of prim. and sec. anilines.

Introduction of bromine into the ortho- and para-positions of an alkyilaniline causes an increase in retention in the reversed-phase LC system. There is a semi-linear relationship between k' values of brominated and non-brominated anilines, which is of value for identification purposes.

In the straight-phase LC system, para-bromination generally causes an increase in retention and ortho-bromination a decrease. As for the reversed-phase system, there is a semi-linear relationship between k' values of brominated and original anilines.

ACKNOWLEDGEMENT

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CAPACITY FACTORS, k' , AND SEPARATION FACTORS, α , FOR ORTHO- AND PARA-ALKYL-SUBSTITUTED ANILINES AND THEIR BROMINATION PRODUCTS IN THE REVERSED-PHASE LC SYSTEM

Column: Nucleosil C₁₈. Eluent: methanol-aqueous buffer (80:20 and 70:30*, v/v, pH = 7.0).

No. Aniline	k'	α									
		(Substituent)		Non-brominated		Tri-brominated		Tri-/di-			
		Non-brominated	Monobrominated	Dibrominated	Tri-brominated	Mono-/non-Ortho Para	Di-/mono-ortho-Ortho-para	Di-/mono-para-Ortho-para	Tri-/di-		
1	None*	0.55	1.33	1.16	3.33	8.87	2.42	2.11	2.50	2.87	2.66
1	None	0.41				3.48					
2	2-Methyl*	0.85	2.20	1.82	5.68		2.59	2.14	2.58	3.12	
2	2-Methyl	0.55	1.10	0.96	2.35		2.00	1.75	2.14	2.45	
3	2-Ethyl*	1.26	3.14	2.54	7.87		2.49	2.02	2.51	3.10	
4	2-Isopropyl*	1.69	4.20	3.33	10.2		2.49	1.97	2.43	3.06	
5	4-Methyl*	0.86	2.01		5.36		2.34		2.67		
6	4-Ethyl*	1.26	3.04		8.25		2.41		2.71		
7	4-Isopropyl*	1.80	4.08		10.8		2.27		2.65		
8	4-n-Butyl*	3.29	7.84		21.5		2.38		2.74		
9	2,4-Dimethyl*	1.25	3.36				2.69				
10	2-Methyl-4-butyl*	5.02	12.7				2.53				
11	2,6-Dimethyl*	1.31		2.89			2.21				
11	2,6-Dimethyl	0.78		1.39			1.78				
12	N-Methyl	0.68		1.25	3.04	5.14	1.84			2.43	1.69
13	N-Ethyl	0.87		1.57	4.39	7.18	1.80			2.80	1.64
14	N-Propyl	1.23		2.26	6.46	11.3	1.84			2.86	1.75
15	N-n-Butyl	1.69		2.79	8.87	15.3	1.65			3.18	1.72
16	N-Phenyl	1.52	3.20	2.79	4.89 [†]	4.71 [§]	2.11	1.84			
17	N-Benzyl	1.50		2.61	6.55	12.3	1.74			2.51	1.88

18	N-Methyl-2-methyl	0.97	1.83	3.80		1.89	2.08
19	N-Ethyl-2-methyl	1.34	2.48	5.30		1.85	2.14
20	N-Ethyl-2-ethyl	1.86	3.23	6.90		1.74	2.14
21	N-Methyl-4-methyl	0.90	2.17	2.95		2.41	1.36
22	N-Ethyl-4-methyl	1.15	3.26	4.20		2.83	1.29
23	N,N-Dimethyl	1.35	2.45		5.02	1.81	1.49
24	N,N-Diethyl	2.32	4.02			1.73	1.76
25	N,N-Diphenyl	7.40	13.0	21.4 ^{\$}	31.9 ⁺	1.76	1.65
26	N,N-Dimethyl-						1.38
	-2-methyl	1.76	3.20	8.91		1.82	2.78
27	N,N-Diethyl-						
	-2-methyl	3.45	6.40	16.2		1.86	2.53
28	N,N-Diethyl-						
	-2-ethyl	5.21	8.94	21.8		1.72	2.44
29	N,N-Dimethyl-						
	-4-methyl	1.80	2.32	7.37		1.29	3.18
30	N,N-Diethyl-						
	-4-methyl	2.95	4.61	13.5		1.56	2.93
31	N,N-Dimethyl-						
	-2,6-dimethyl	4,20	7.84			1.87	

⁺ 2,4'-dibrominated

^{\$} 4,4'-dibrominated

[#] 2,4,4'-tribrominated; 2,4,2',4'-tetrabrominated; k' = 2.03

[†] 4,4',4''-tribrominated

TABLE II

CAPACITY FACTORS, k' , AND SEPARATION FACTORS, α , FOR ORTHO- AND PARA-ALKYL-SUBSTITUTED ANILINES AND THEIR BROMINATION PRODUCTS IN THE STRAIGHT-PHASE LC SYSTEM

Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane.

No. Aniline	(Substituent)	k'		α						
		Non-brominated	Monobrominated Ortho Para	Dibrominated Di-ortho Ortho-para	Tri-brominated	Mono-/non- Ortho Para	Di-/mono-ortho- Di-ortho Ortho-para	Di-/mono-para- Ortho-para	Tri-/di-	
1	None	7.53	2.65 10.7	3.96	0.87	0.35 1.42	1.49	0.37	0.22	
2	2-Methyl	4.84	1.41 6.74	2.06		0.29 1.39	1.46	0.31		
3	2-Ethyl	3.65	1.13 5.20	1.57		0.31 1.42	1.39	0.30		
4	2-Isopropyl	3.14	0.93 4.45	1.28		0.30 1.42	1.38	0.29		
5	4-Methyl	9.62	2.50		0.67	0.26	0.27			
6	4-Ethyl	8.72	2.26		0.60	0.26	0.27			
7	4-Isopropyl	8.87	2.16		0.57	0.24	0.26			
8	4- <u>n</u> -Butyl	8.72	2.11		0.54	0.24	0.26			
9	2,4-Dimethyl	6.04	1.36			0.23				
10	2-Methyl-4-butyl	5.07	1.20			0.24				
11	2,6-Dimethyl	2.83	3.91			1.38				
12	N-Methyl	2.32	3.19	0.87	0.42	1.38		0.27	0.48	
13	N-Ethyl	1.49	1.98	0.51	0.29	1.33		0.26	0.57	
14	N-Propyl	1.06	1.50	0.41	0.25	1.42		0.27	0.61	
15	N- <u>n</u> -Butyl	0.90	1.27	0.37	0.23	1.41		0.29	0.62	
16	N-Phenyl	2.50	0.61 6.89	0.87 ⁺ 4.01 [§]	1.04 [‡]	0.24 2.76				
17	N-Benzyl	1.65	2.62	1.43	0.43	1.59		0.55	0.30	
18	N-Methyl-2-methyl	1.43	2.01	0.58		1.41		0.29		
19	N-Ethyl-2-methyl	0.82	1.11	0.39		1.35		0.35		

Continued

TABLE II. continued

20	N-Ethyl-2-ethyl	0.67		0.90	0.32		1.34		0.36
21	N-Methyl-4-methyl	2.73	0.61	0.53			0.22	0.87	
22	N-Ethyl-4-methyl	1.72	0.39	0.38			0.23	0.97	
23	N,N-Dimethyl	0.65		0.73	0.44	0.14	1.12		0.60
24	N,N-Diethyl	0.50		0.48	0.24		0.96		0.50
25	N,N-Diphenyl	0.39		0.52	0.46 ^{\$}	0.41 [‡]	1.33		0.88
26	N,N-Dimethyl-								0.32
	-2-methyl	0.45		0.42	0.15		0.93		0.36
27	N,N-Diethyl-								
	-2-methyl	0.29		0.22	0.13		0.76		0.59
28	N,N-Diethyl-								
	-2-ethyl	0.21		0.18	0.11		0.86		0.61
29	N,N-Dimethyl-								
	-4-methyl	0.79	0.47	0.18			0.59	0.38	
30	N,N-Diethyl-								
	-4-methyl	0.90	0.24	0.15			0.27	0.63	
31	N,N-Dimethyl-								
	-2,6-dimethyl	0.14		0.18			1.29		

[‡]2,4'-dibrominated^{\$}4,4'-dibrominated[#]2,4,4'-tribrominated; 2,4,2',4'-tetrabrominated; k' = 0.44[‡]4,4',4''-tribrominated

TABLE III

COMPARISON OF CAPACITY FACTORS, k' , FOR BROMINATED PRIM., SEC. AND TERT. ALKYLANILINES IN THE STRAIGHT-PHASE LC SYSTEM

Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane.

Substituent	Prim. anilines	Sec. anilines	Tert. anilines
Ortho-bromo	0.93 - 2.65	0.39 - 0.61	0.24 - 0.47
Para-bromo	4.45 - 10.7	0.90 - 3.19	0.18 - 0.73
Di-ortho-bromo	0.54 - 0.67	0.38 - 0.53	0.15 - 0.18
Ortho-para-di-bromo	1.28 - 3.96	0.32 - 0.87	0.11 - 0.44
Tri-bromo	0.87	0.23 - 0.42	0.14

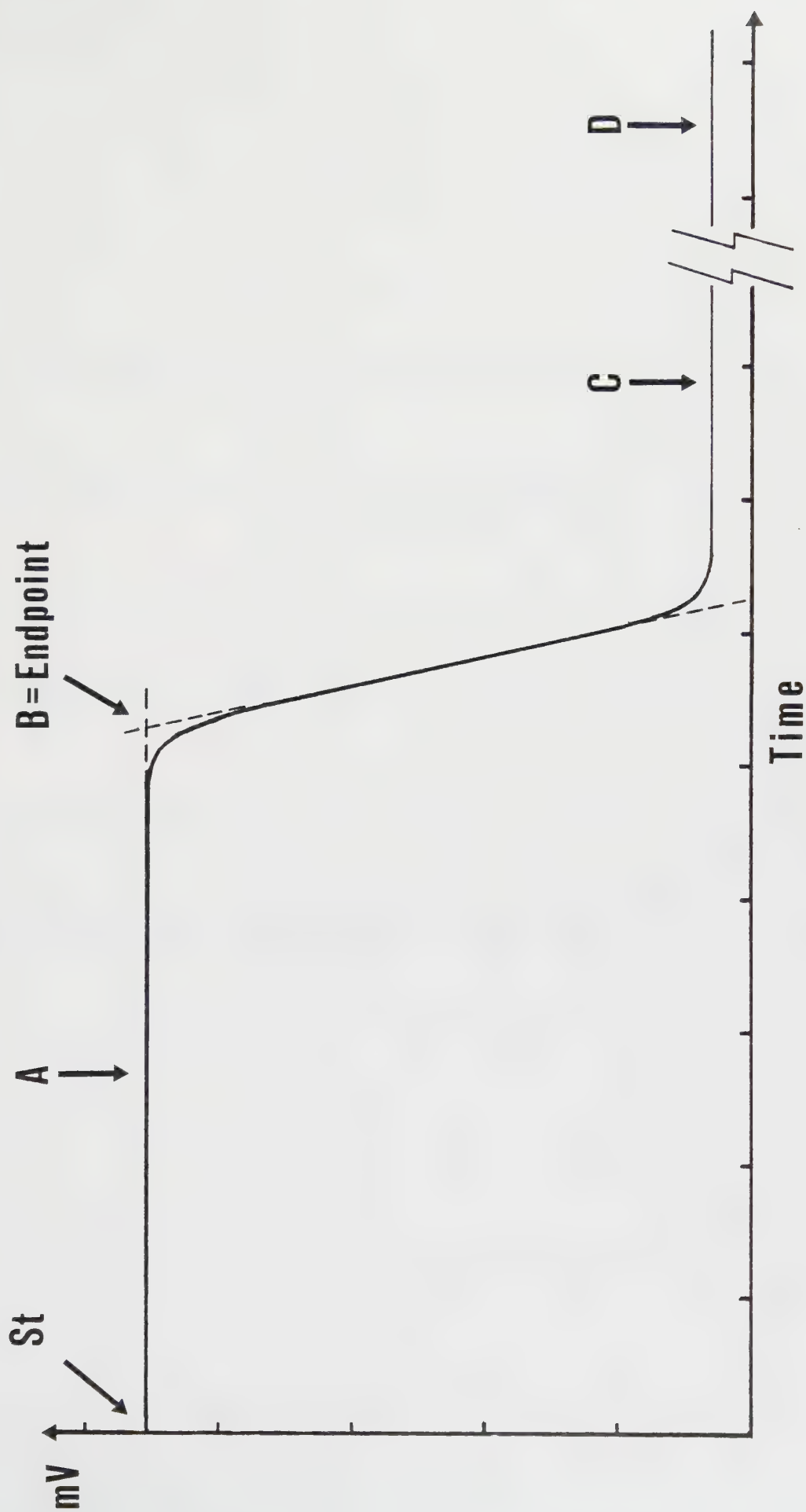


Fig. 1. Titration curve for coulometric bromination of alkylanilines. St, A, B, C and D indicate points at which samples were removed from the titration vessel.

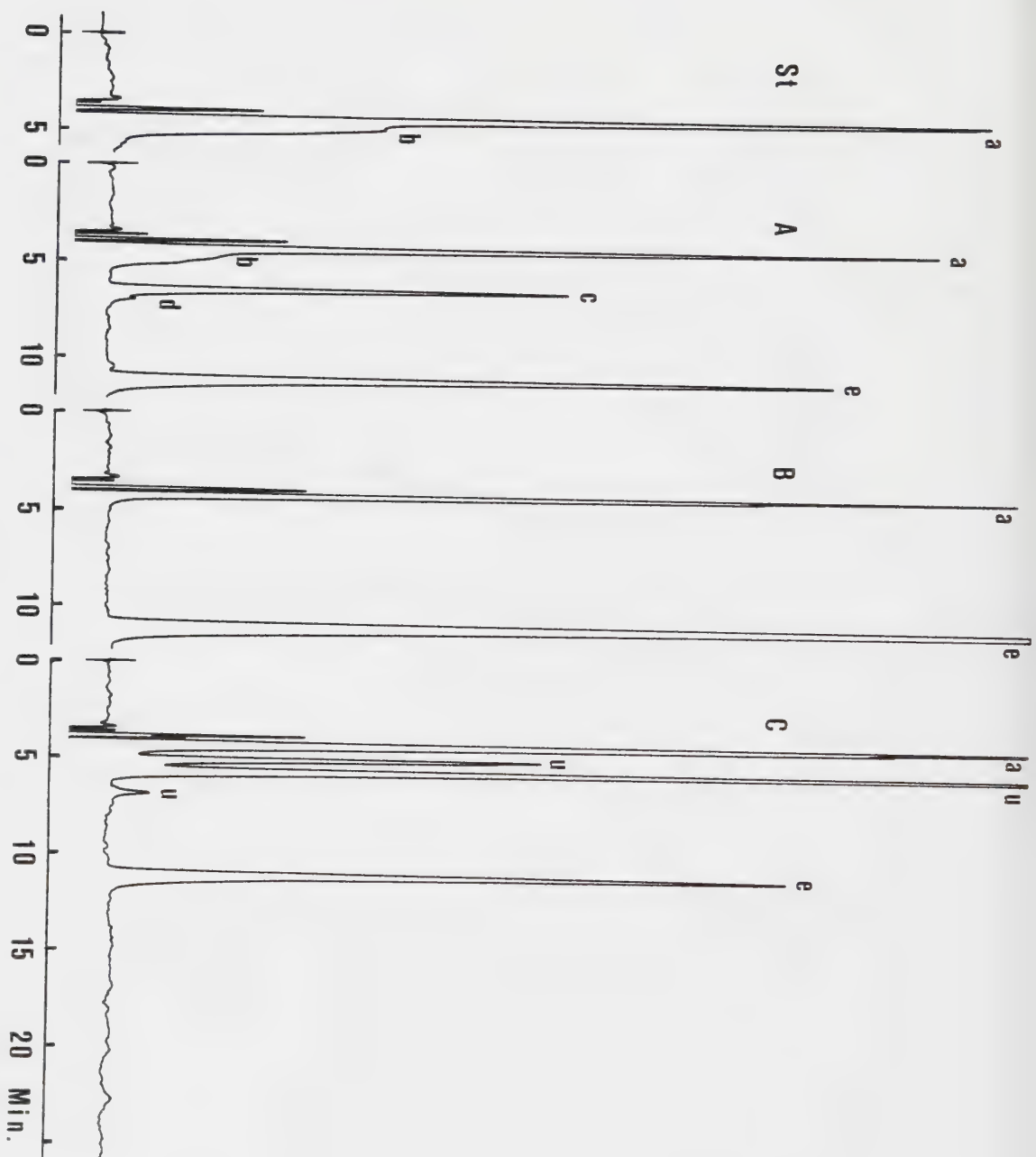


Fig. 2. Chromatograms of the product mixture after bromination of 2-methylaniline. Column: Nucleosil C_{18} . Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml $\cdot$ h $^{-1}$. Wavelength: 280 nm. Volume injected: 20 μ l. Peaks: a = acetic acid; b = 2-methylaniline; c = 4-bromo-2-methylaniline; d = 6-bromo-2-methylaniline; e = 4,6-dibromo-2-methylaniline; u = unknown compound. St, A, B and C refer to Fig. 1.

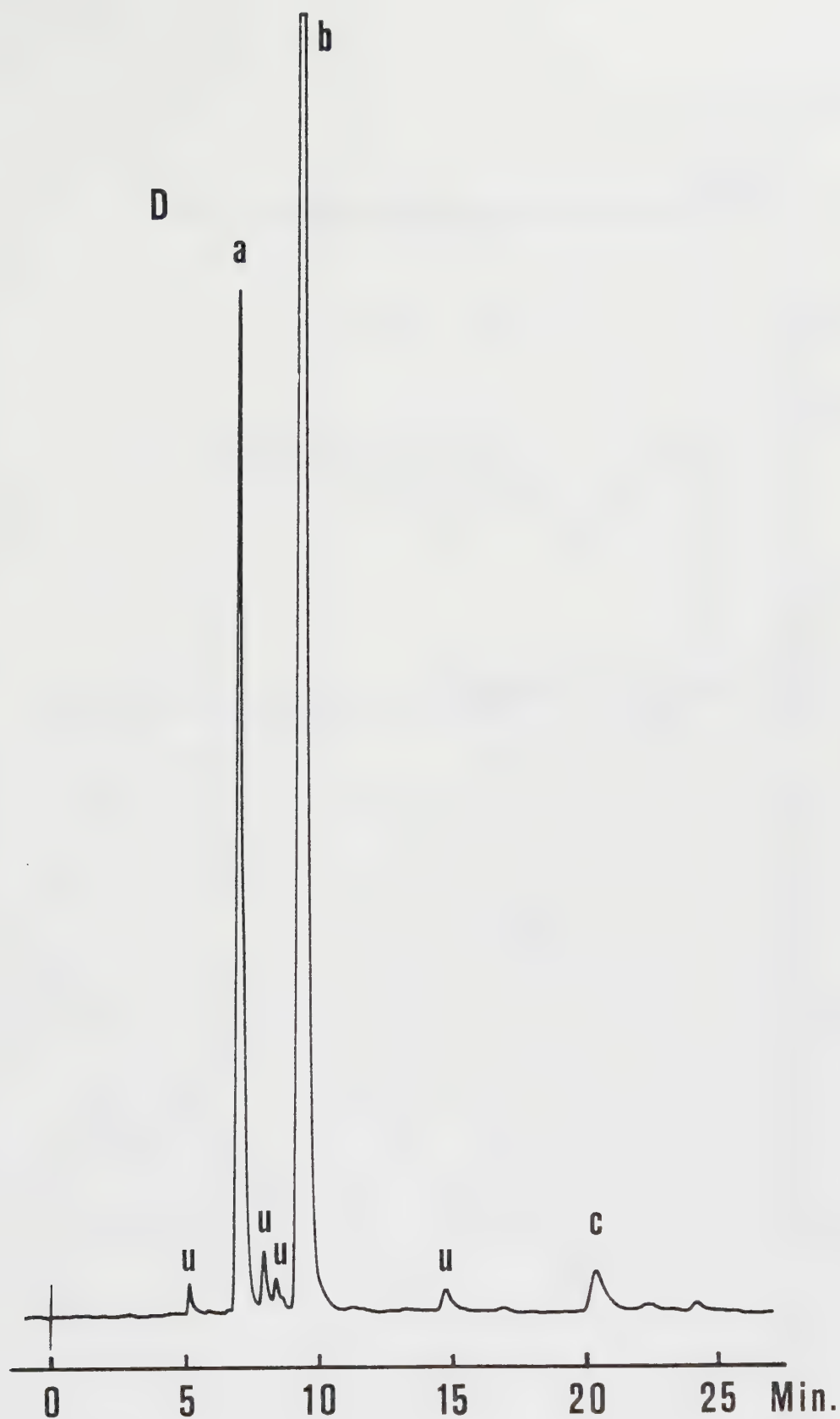


Fig. 3. Chromatogram of the product mixture after overbromination of 2,6-dimethylaniline. Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane; $45 \text{ ml} \cdot \text{h}^{-1}$. Wavelength: 280 nm. Volume injected: 20 μl . Peaks: a = 4,4'-dibromo-2,6,2',6'-tetramethylhydrazobenzene; b = 4-amino-4'-bromo-3,5,2',6'-tetramethyldiphenylamine; c = 4-bromo-2,6-dimethylaniline; u = unknown compounds. D refers to Fig. 1.

B

D

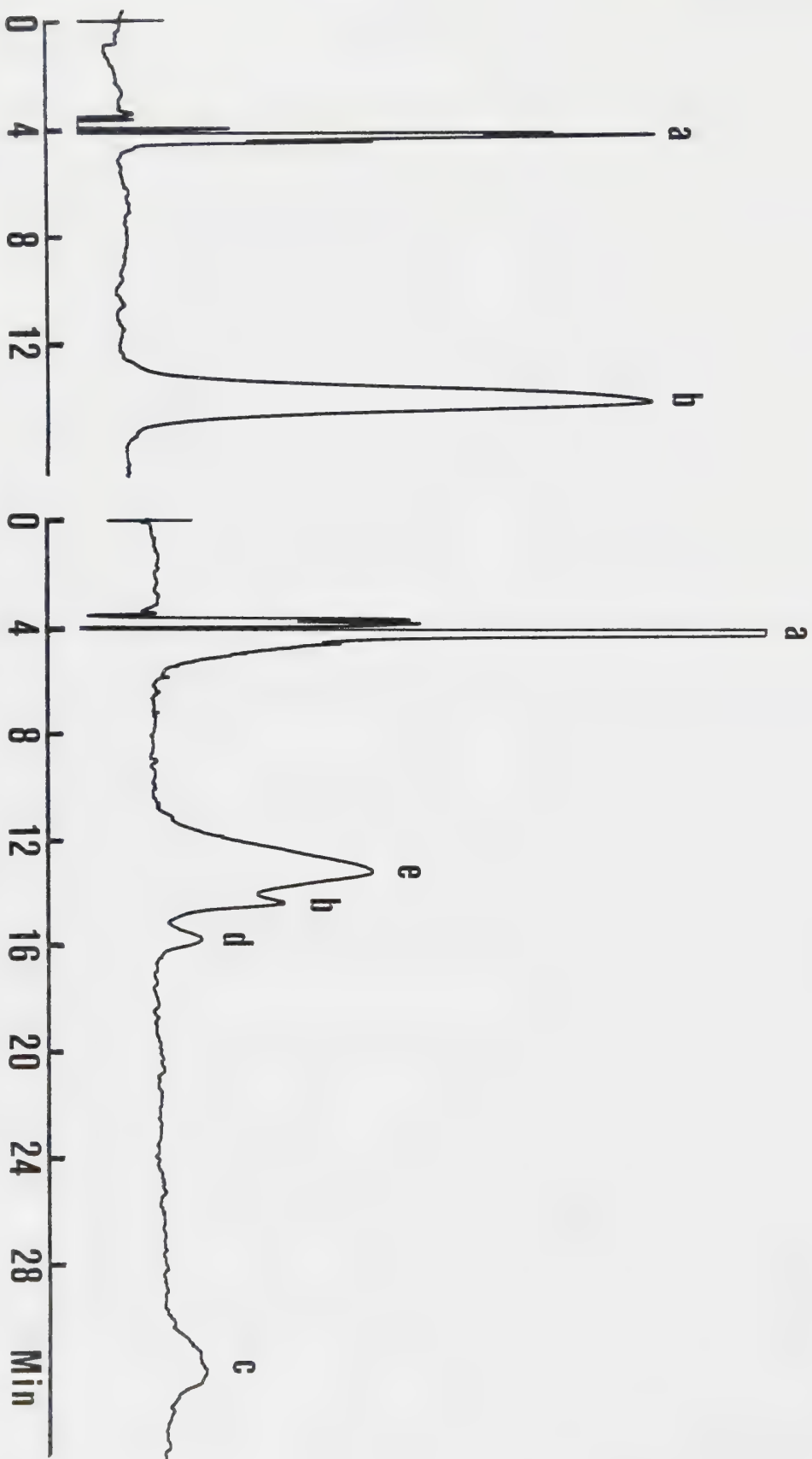


Fig. 4. Chromatogram of the product mixture after bromination of N,N-dimethyl-2-methylaniline. Column: Nucleosil C₁₈. Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml · h⁻¹. Wavelength: 280 nm. Volume injected 20 µl. Peaks: a = acetic acid; b = N,N-dimethyl-2-methyl-4-bromoaniline; c = N,N-dimethyl-2-methyl-4,6-dibromoaniline; d = N-methyl-2-methyl-4,6-dibromoaniline; e = 2-methyl-4,6-dibromoaniline. B and D refer to Fig. 1.



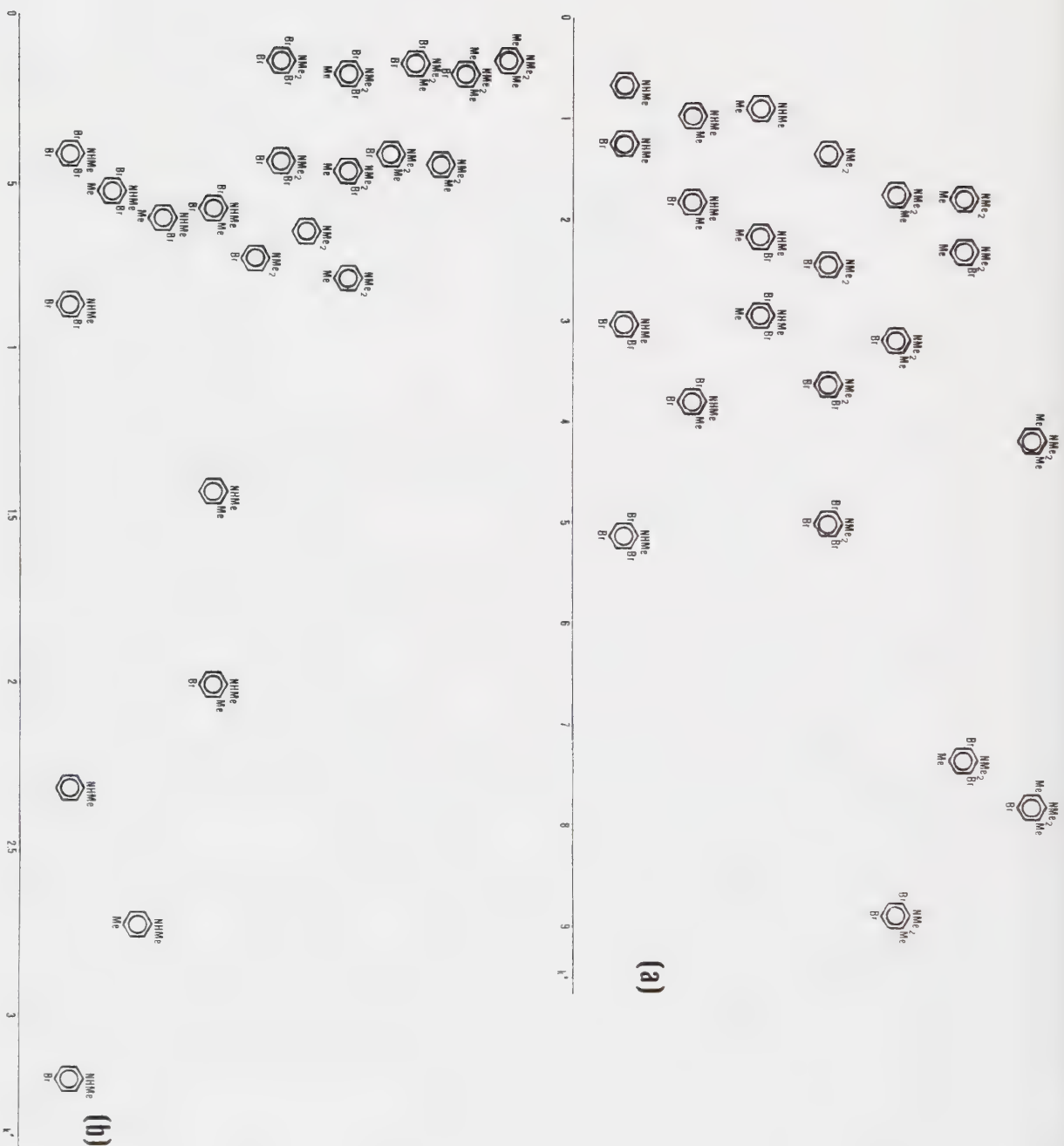




Fig. 7. Relationship between capacity factor, k' , for alkydanilines and the corresponding bromination products in reversed-phase LC. Column: Nucleosil C_{18} . Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml $\cdot$ h $^{-1}$. ■ = mono-para-brominated; ● = mono-ortho-brominated; o = ortho-para-brominated; ▽ = di-ortho-brominated; □ = tri-brominated.

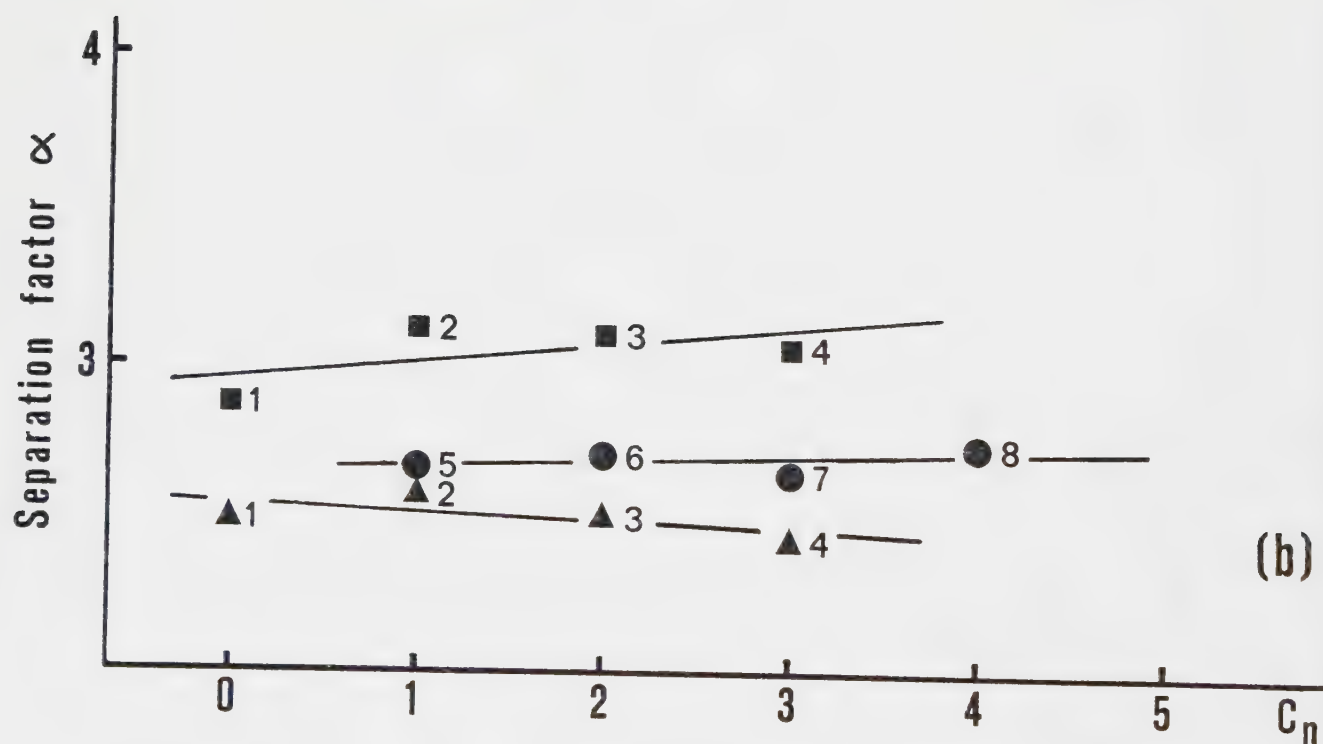
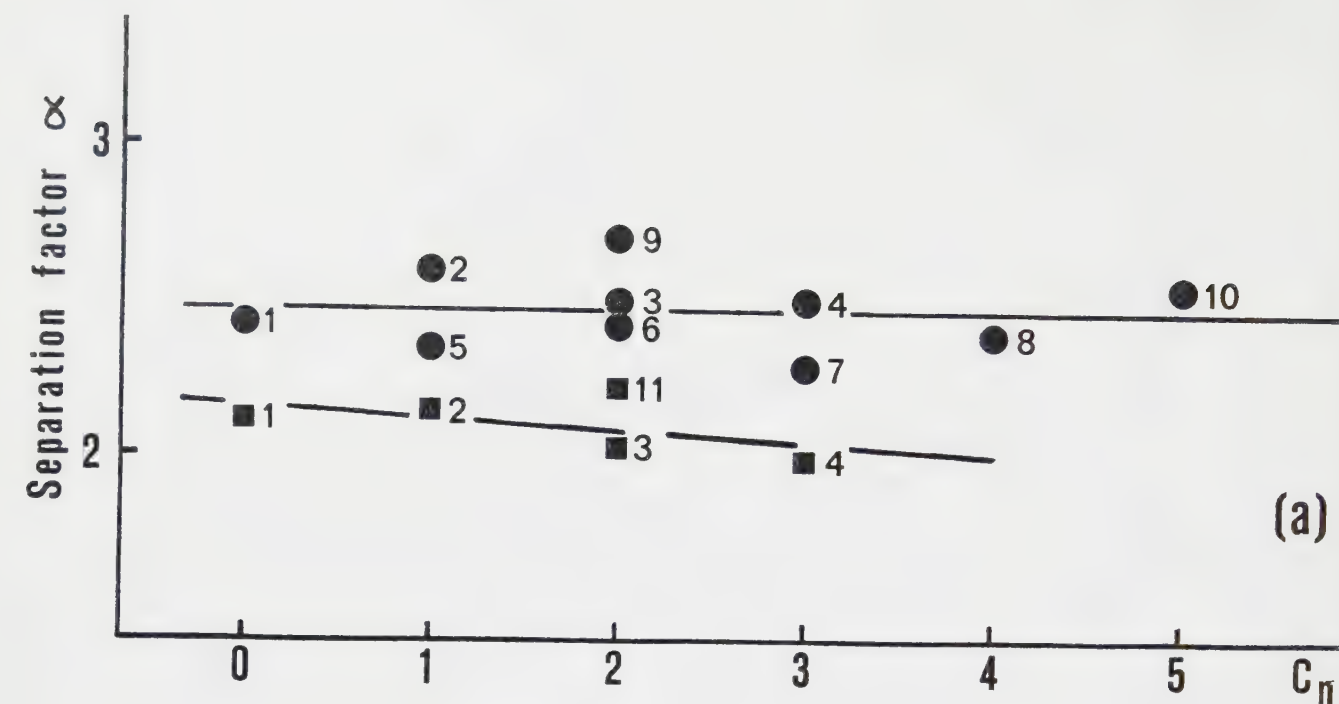


Fig. 8. Relationship between the separation factor, α , for monobrominated to non-brominated and for dibrominated to monobrominated prim. alkyylanilines, and alkyl carbon number. Column: Nucleosil C_{18} . Eluent: methanol-aqueous buffer (70:30, v/v, pH = 7.0); $45 \text{ ml} \cdot \text{h}^{-1}$. (a) Mono-/non-brominated prim. alkyylaniline; $\blacksquare$ = para-/non-brominated; $\bullet$ = ortho-/non-brominated. (b) Di-/monobrominated prim. alkyylaniline; $\blacktriangle$ = ortho-para-/ortho-brominated; $\bullet$ = di-ortho-/ortho-brominated; $\blacksquare$ = ortho-para-/para-brominated. The numbers refer to Table I.

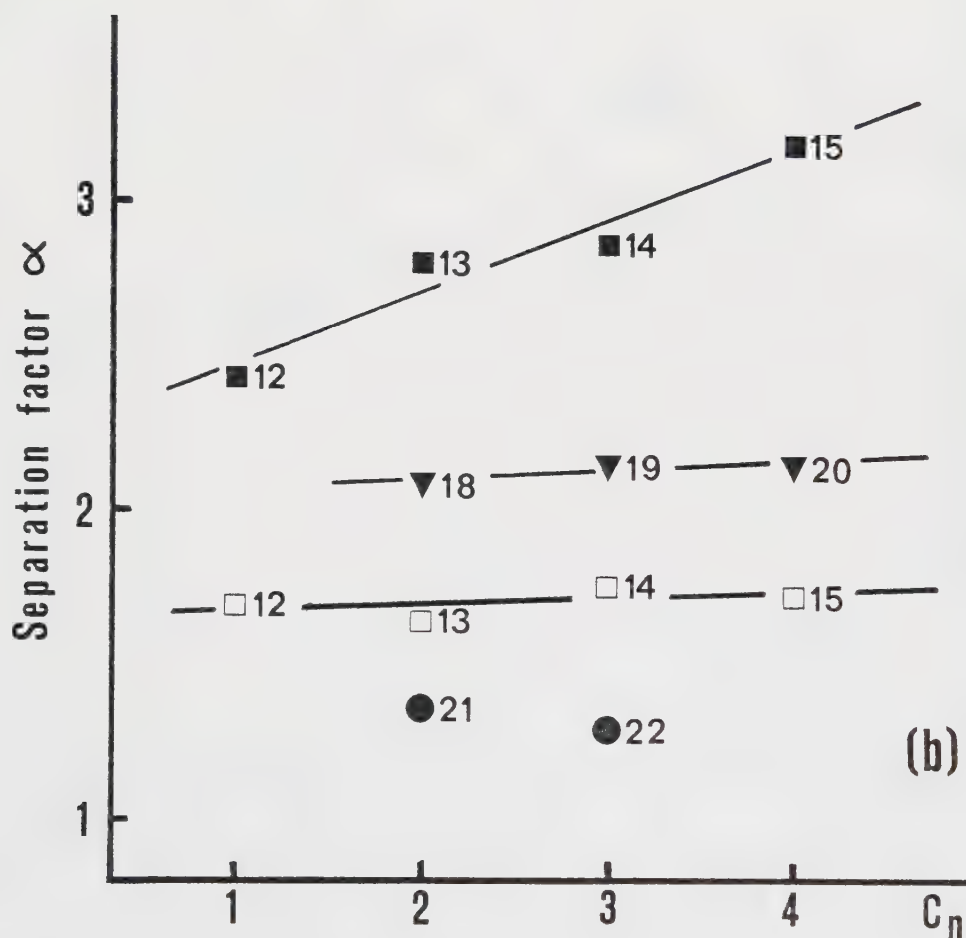
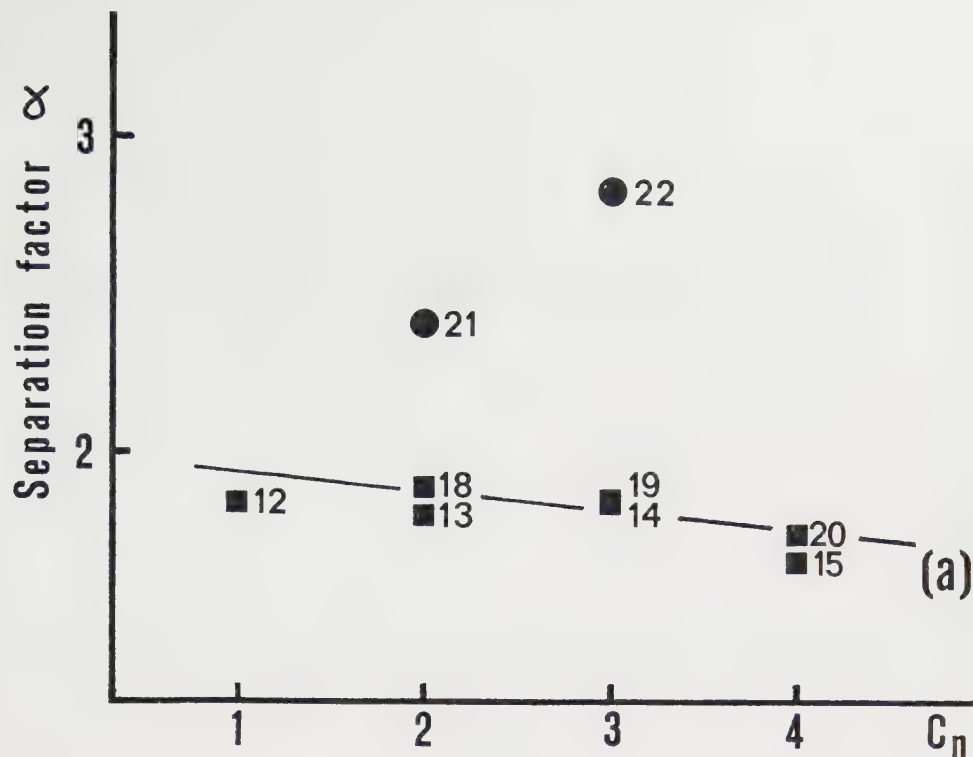


Fig. 9. Relationship between the separation factor, α , for monobrominated to non-brominated and dibrominated to monobrominated sec. alkylanilines, and alkyl carbon number. Column: Nucleosil C_{18} . Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0); $45 \text{ ml} \cdot \text{h}^{-1}$. (a) Mono-/non-brominated sec. alkylaniline; ■ = para-/non-brominated; ● = ortho-/non-brominated. (b) di-/monobrominated and tri-/ortho-para-brominated sec. alkylanilines; ● = di-ortho-/ortho-brominated; ▼ = ortho-para-/para-brominated (ortho-alkyl-substituted); ■ = ortho-para-/para-brominated (non-ortho-alkyl-substituted); □ = tri-/ortho-para-brominated. The numbers refer to Table I.

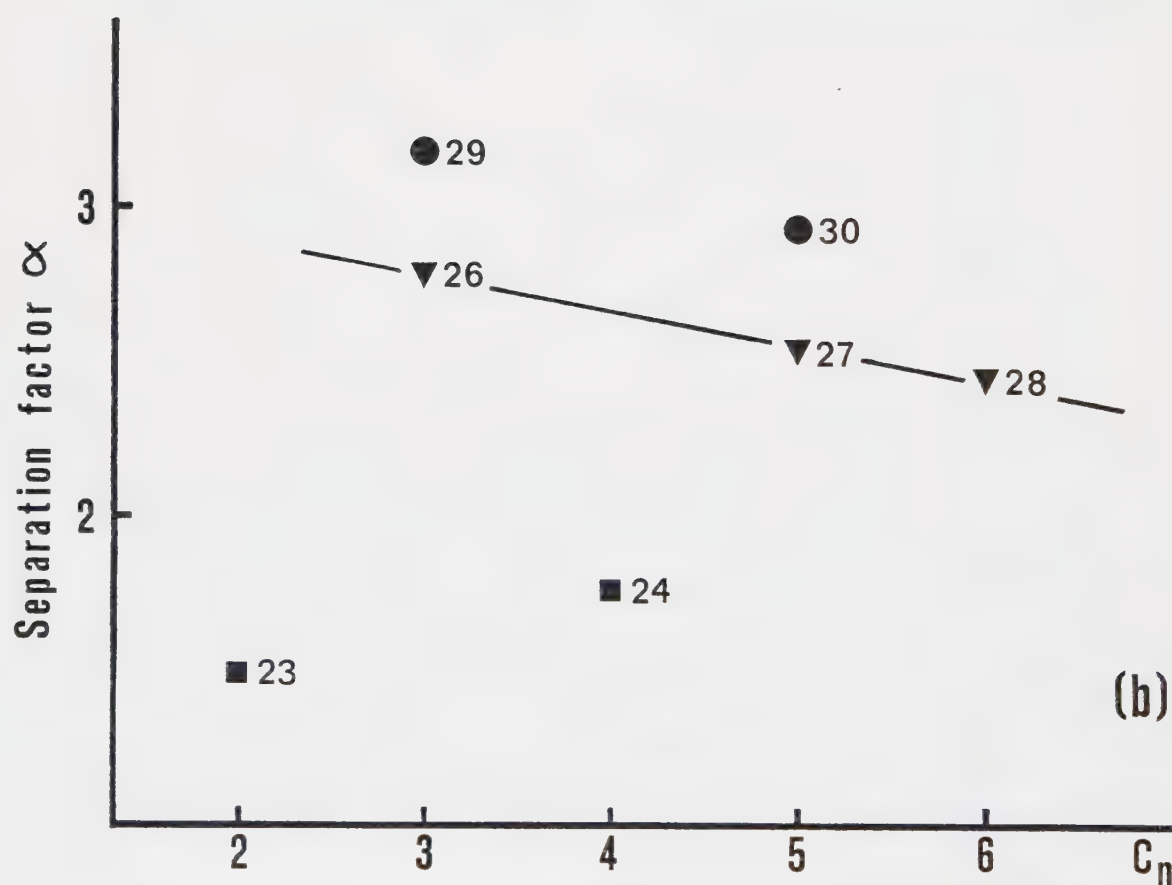
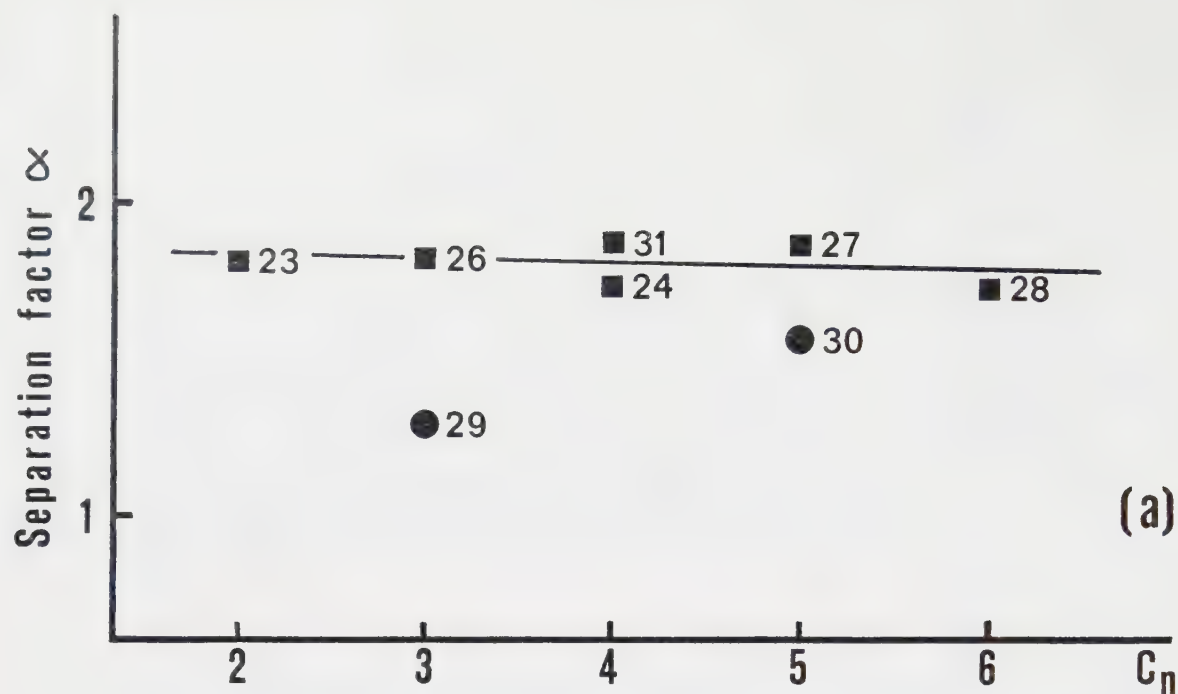


Fig. 10. Relationship between the separation factor, α , for monobrominated to non-brominated and dibrominated to monobrominated tert. alkylanilines, and alkyl carbon number. Column: Nucleosil C_{18} . Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0); $45 \text{ ml} \cdot \text{h}^{-1}$. (a) Mono-/non-brominated tert. alkylaniline; ● = ortho-/non-brominated; ■ = para-/non-brominated. (b) Di-/monobrominated tert. alkylaniline; ■ = ortho-para-/para-brominated (non-ortho-alkyl-substituted); ▼ = ortho-para-/para-brominated (ortho-alkyl-substituted); ● = di-ortho-/ortho-brominated. The numbers refer to Table I.

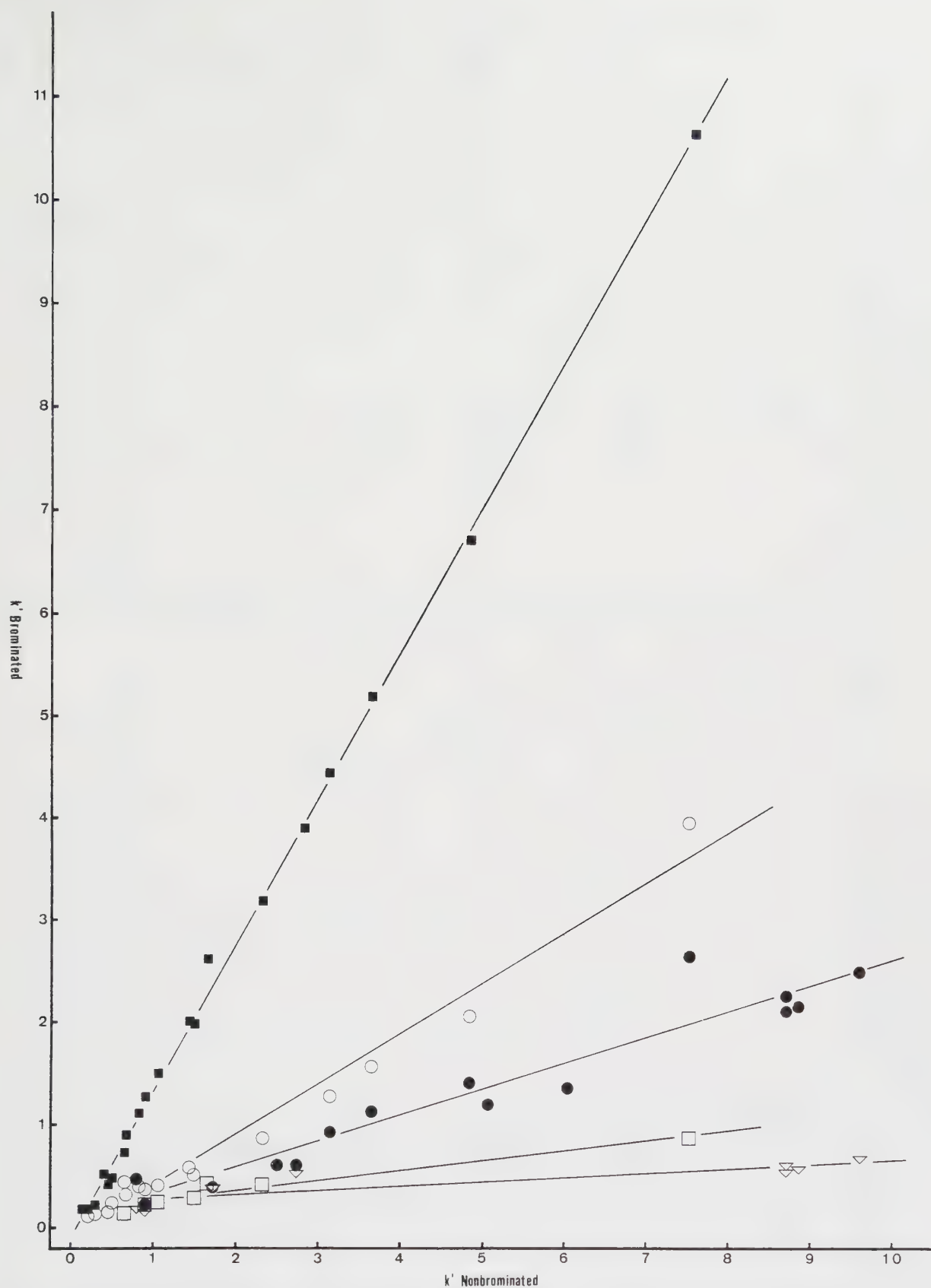


Fig. 11. Relationship between capacity factor, k' , for alkylanilines and the corresponding bromination products in straight-phase LC. Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane; $45 \text{ ml} \cdot \text{h}^{-1}$. ■ = mono-para-brominated; ● = mono-ortho-brominated; ○ = ortho-para-brominated; ▽ = di-ortho-brominated; □ = tribrominated.

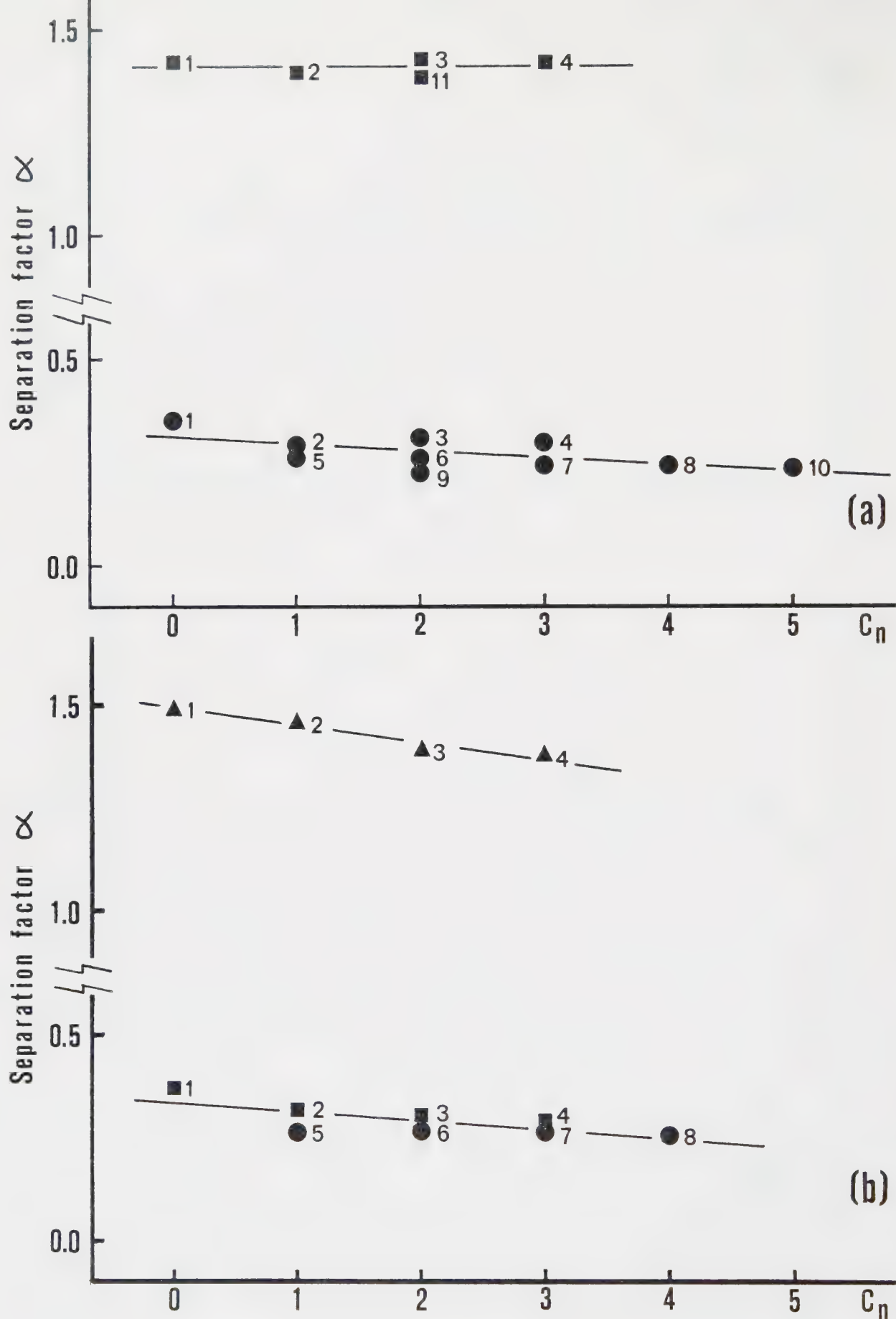


Fig. 12. Relationship between the separation factor, α , for monobrominated to non-brominated and dibrominated to monobrominated prim. alkylanilines, and alkyl carbon number. Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane; $45 \text{ ml} \cdot \text{h}^{-1}$. (a) Mono-/non-brominated prim. alkylaniline; $\bullet$ = ortho-/non-brominated; $\blacksquare$ = para-/non-brominated. (b) Di-/monobrominated prim. alkylaniline; $\bullet$ = di-ortho-/ortho-brominated; $\blacksquare$ = ortho-para-/para-brominated; $\blacktriangle$ = ortho-para-/ortho-brominated. The numbers refer to Table II.

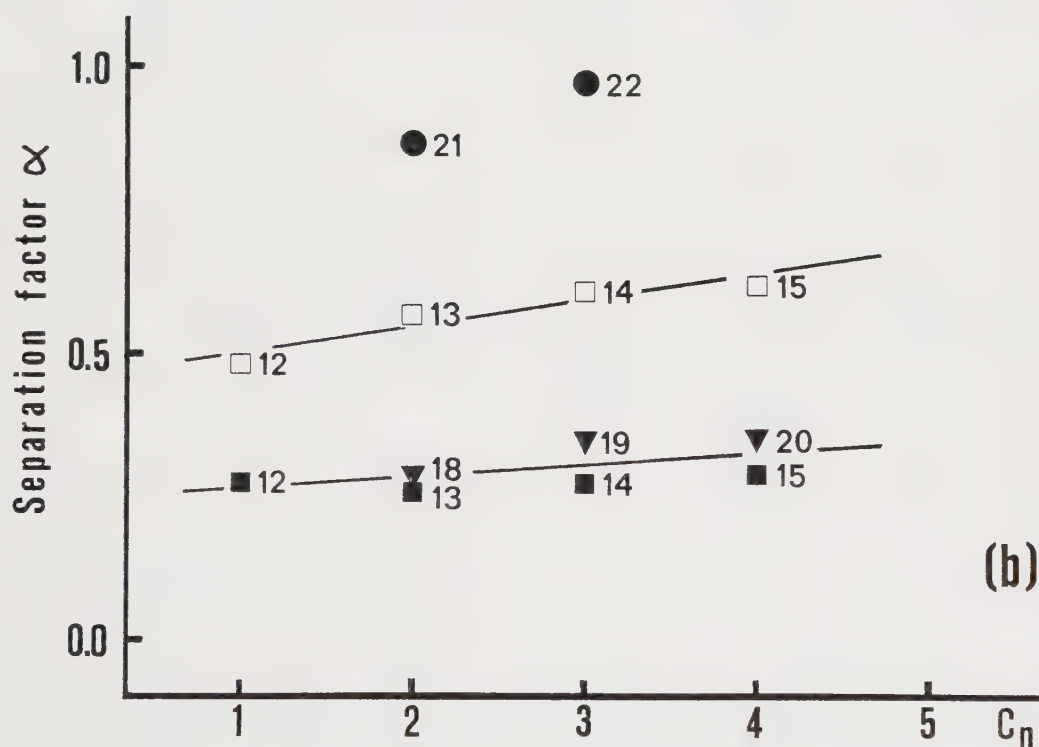
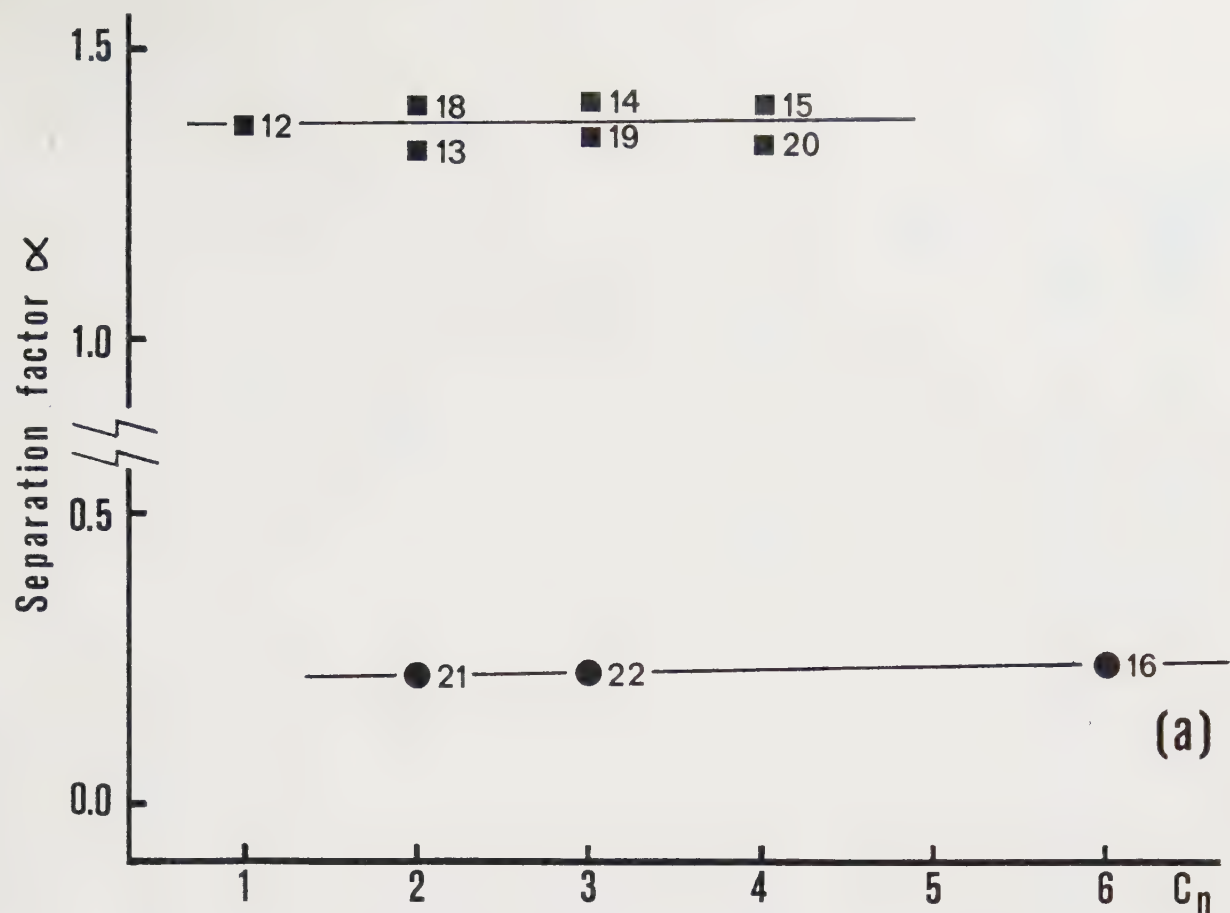


Fig. 13. Relationship between the separation factor, α , for monobrominated to non-brominated and dibrominated to monobrominated sec. alkylanilines, and alkyl carbon number. Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane; $45 \text{ ml} \cdot \text{h}^{-1}$. (a) Mono-/non-brominated sec. alkylaniline; ● = ortho-/non-brominated; ■ = para-/non-brominated. (b) Di-/monobrominated and tri-/ortho-para-brominated sec. alkylanilines; ● = di-ortho-/ortho-brominated; ■ = ortho-para-/para-brominated (non-ortho-alkyl-substituted); ▼ = ortho-para-/para-brominated (ortho-alkyl-substituted); □ = tri-/ortho-para-brominated. The numbers refer to Table II.

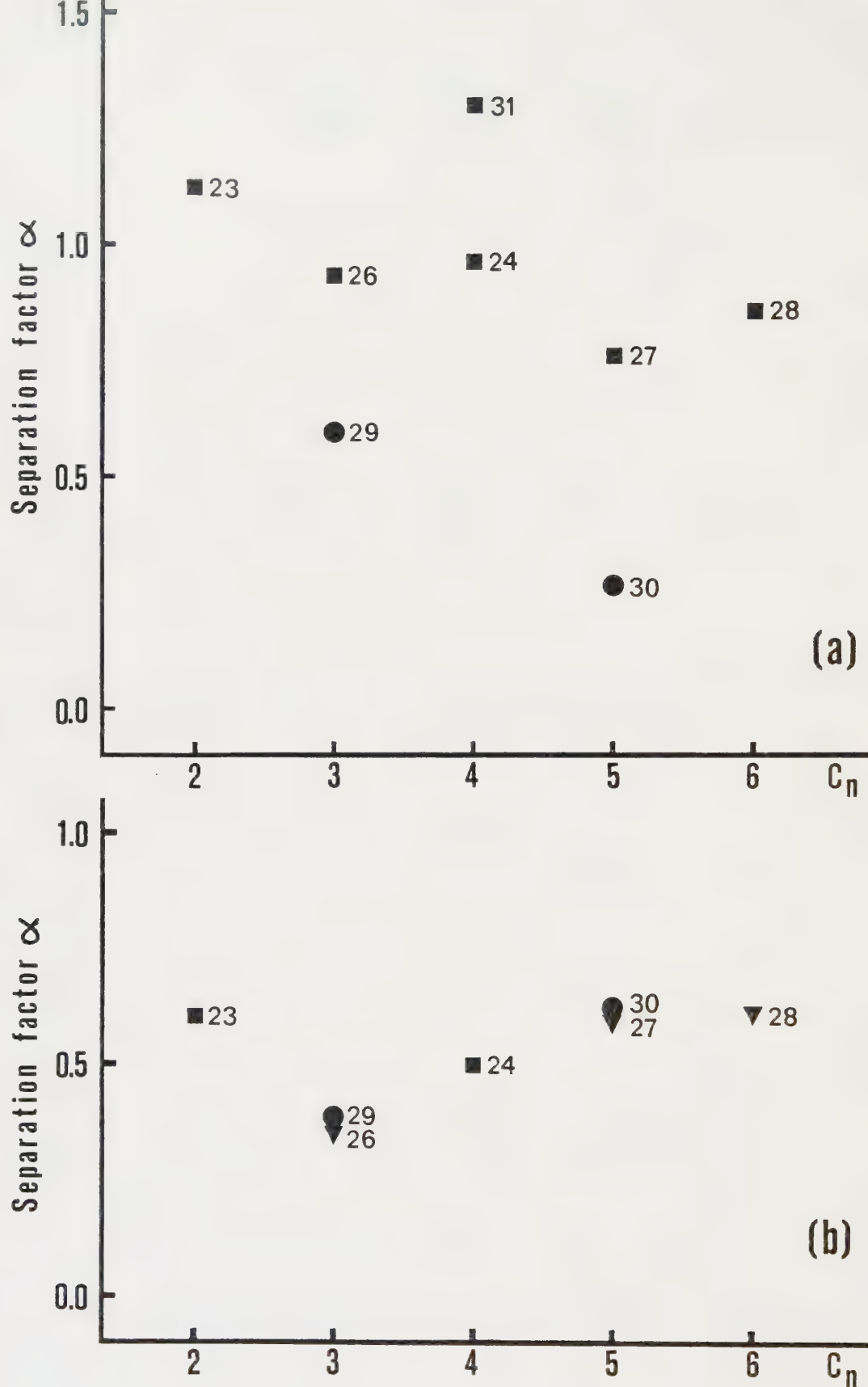


Fig. 14. Relationship between the separation factor, α , for monobrominated to non-brominated and dibrominated to monobrominated tert. alkylanilines, and alkyl carbon number. Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane; $45 \text{ ml} \cdot \text{h}^{-1}$. (a) Mono-/non-brominated tert. alkylaniline; $\bullet$ = ortho-/non-brominated; $\blacksquare$ = para-/non-brominated. (b) Di-/monobrominated tert. alkylaniline; $\bullet$ = di-ortho-/ortho-brominated; $\blacksquare$ = ortho-para-/para-brominated (non-ortho-alkyl-substituted); $\blacktriangledown$ = ortho-para-/para-brominated (ortho-alkyl-substituted). The numbers refer to Table II.

V

LIQUID CHROMATOGRAPHY STUDY OF BROMINATED ANILINES AND INVESTIGATION
OF PRODUCT FORMATION IN THE BROMINATION REACTION

II. ANILINES WITH ALKYL GROUPS IN THE META-POSITION

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SUMMARY

Straight- and reversed-phase liquid chromatography (LC) have been used to study product formation in the quantitative coulometric bromination of the headlined anilines. The coulometric method yields up to the end-point predictable, unambiguous products by exchange of hydrogen for bromine in free ortho- and para-positions. After the end-point, oxidation products may be formed from prim. anilines and N-alkyl groups split off from sec. and tert. anilines.

A detailed study of retention behaviour of some 30 bromoanilines in straight- and reversed-phase LC has been made. It turned out that an increase in retention generally took place on the introduction of bromine into the aniline nucleus, but for the formation of o-bromoanilines in straight-phase LC, where retention decreased. A semi-linear relationship was found to exist between capacity factors of brominated and non-brominated anilines in both of the LC systems.

INTRODUCTION

In a previous paper¹ product formation in the quantitative coulometric bromination of anilines with alkyl groups in the ortho- and para-positions was studied and retention behaviour of the formed bromoanilines in straight- and reversed-phase liquid chromatography (LC) investigated. The present work is a continuation of this study applied to meta-alkyl-substituted anilines.

EXPERIMENTAL

The experimental conditions were described in detail in the previous paper in this series¹. In order to identify certain of the anilines formed on bromination 3-methyl-4,6-dibromoaniline, 3-ethyl-6-bromoaniline, N-ethyl-3-methyl-4,6-dibromoaniline, N,N-dimethyl-3-methyl-4,6-dibromoaniline and N,N-diethyl-3-methyl-4,6-dibromoaniline were prepared at this laboratory.

RESULTS AND DISCUSSION

Choice of liquid chromatographic system

For the chromatographic study were used one reversed-phase octadecylsilane (C₁₈) system with methanol-aqueous buffer (80:20, v/v, pH = 7.0) as eluent and one straight-phase system on nitrile phase with isooctane containing 0.2% (v/v) 2-propanol as eluent.

Product formation in coulometric bromination

In the coulometric bromination method for the titration of anilines described by Truedsson and Smith², the reaction is carried out in a water-acetic acid medium and the reactivity is controlled by varying the water content and the bromide ion concentration and by the addition of pyridine. The reaction is believed to involve substitution with bromine at free ortho- and para-positions. For anilines with several such positions, the titration can be carried out to either the fully brominated stage or to a stage corresponding to the introduction of a smaller number of bromine atoms than the number of available free

ortho- and para-positions. For a certain aniline, the outcome of a titration is dependent both on the structure and on the bromination-promoting properties of the titration medium.

In the present investigation the aim was to study product formation during titration in the pyridine-free medium III-1, containing acetic acid and water in the proportions 60:40 (v/v) and with a bromide concentration of 0.1 mole l^{-1} . For this purpose samples were removed from the titration vessel at various stages of the titration and injected on to the LC column either directly (C_{18} phase) or after extraction (nitrile phase). A typical titration curve, with points of sample removal indicated, is given in ref. 1 Fig. 1.

At the quantitative bromination in medium III-1 (point B on the titration curve), prim. anilines studied in this work consume bromine corresponding to the number of free ortho- and para-positions, sec. anilines consume one bromine atom less and tert. anilines two bromine atoms less². From the chromatograms of the samples taken at the end-point B, it was established that e.g. 3-methylaniline was converted to 2,4,6-tribromo-3-methylaniline, N-methyl-3-methylaniline to a mixture of the 2,4- and 4,6-dibromo-derivatives and N,N-dimethyl-3-methylaniline to the 4-bromo-derivative.

Fig. 1 illustrates the bromination course for N-methyl-3-methylaniline. At point A, half-way to the end-point, the main bromination products formed are N-methyl-3-methyl-4-bromoaniline (b) and the corresponding 2,4- and 4,6-dibromo-derivatives (c). In addition, a small amount of N-methyl-3-methyl-2,4,6-tribromoaniline (d) is present. At the end-point B only the mixture of the dibromo-derivatives and somewhat of the tribromo-derivative are present. The formation of the latter compound is in agreement with the slight overconsumption of bromine found for N-methyl-3-methylaniline on quantitative coulometric bromination in medium III-1².

As shown by Table 1, a mono-ortho-brominated derivative is generally not formed on bromination of meta-alkyl-substituted anilines with a free para-position. There is only one example of such a compound being formed, viz. in the case of 3-ethylaniline. It appears, that the steric hindrance, exerted by a more bulky substituent in the meta-position than methyl, decreases the speed of the para-bromination reaction enough to allow the formation of a 3-alkyl-6-bromoaniline.

On dibromination of 3-alkylanilines with free ortho- and para-positions two isomers can be formed, viz. 2,4- and 4,6-dibromo-3-alkylanilines. As shown by Table I, both isomers are formed from prim. and sec. anilines, whereas tert. anilines only yield the 4,6-dibromo-derivative. This fact is undoubtedly caused by increased steric hindrance to bromine substitution at the 2-ortho-position.

Effect of over-bromination. Prim. 3-alkylanilines without ortho-situated alkyl groups are comparatively stable against excess of bromine. At 50% excess (point C on the titration curve), small early peaks appear at separation on the C₁₈ phase. Spectral evidence indicates that their structure is quinoidic.

If the aniline in addition to a meta-standing alkyl group contains an alkyl group in the ortho-position, it becomes more sensitive to an excess of bromine, and quinoidic oxidation products can appear already at the end-point B. At 50% excess of bromine (point C), a considerable amount of the oxidation product has been formed. This is shown for 2,5-dimethylaniline in Fig. 2. Early peaks also appear on the straight-phase system, indicating the formation of substituted diphenylamines, as was reported in the previous paper on ortho-substituted anilines¹.

Sec. and tert. anilines are not full-brominated at the end-point, but still contain vacant ortho-positions. The result of over-bromination is, that these free positions are substituted with bromine, producing the full-brominated aniline (see Table I). However, this reaction cannot be utilized for quantitative purposes. The fully brominated sec. and tert. anilines are very sensitive to further excess of bromine and react with loss of N-alkyl groups to the corresponding prim. and sec. anilines. This is demonstrated in Fig. 1C where N-methyl-3-methylaniline on over-bromination furnishes N-methyl-3-methyl-2,4,6-tribromoaniline (d) and 3-methyl-2,4,6-tribromoaniline (e). In addition a small amount of an oxidation product (f) is formed.

Retention behaviour of brominated anilines

Reversed-phase chromatography. The substitution of bromine into free ortho- and para-positions of meta-alkyl-substituted prim., sec. and tert. anilines gives a considerable increase in retention in reversed-

-phase chromatography (Table I and Fig. 3). There exists a semi-linear relationship between k' values of original anilines on one hand, and k' values of the corresponding mono-, di- and tribrominated derivatives, respectively, on the other hand (Fig. 4). Dibrominated tert. anilines seem to fall on a line of their own (see broken line in Fig. 4).

The effect on retention of the successive introduction of bromine into a meta-alkyl-substituted aniline is demonstrated in Table I, where the separation factors, α , between the various bromination stages are given. As was established already in the previous work on brominated alkyilanilines¹, the sensitivity of the α values to the environmental structure is considerable. This is especially evidenced by their variation in the dibromo- to monobromo- and tribromo- to dibromo- series, respectively.

A kind of brominated anilines, which has not been encountered previously, is tribromoanilines containing a nuclear alkyl group. Comparison of k' values of these compounds with those of corresponding tribromoanilines without a nuclear alkyl group¹ indicates certain anomalies. Thus, it appears that the retention difference is abnormally high between N,N-dimethyl-3-methyl-2,4,6-tribromoaniline and N,N-dimethyl-2,4,6-tribromoaniline, the relationship between the k' values being 3.5. For the corresponding prim. and sec. (N-methyl) aniline pairs the same relationship is only 1.4. Since the anilines, constituting a pair, must be considered to be of similar type, the retention differences between the members in each pair should, following Locke³, be due mainly to solubility differences in the moving phase.

Straight-phase chromatography. A great decrease in retention in straight-phase chromatography for ortho-brominated alkyilanilines in comparison with corresponding non-ortho-brominated compounds, was already established in the previous investigation on brominated anilines from this laboratory¹. Meta-alkyl-substituted anilines behave similarly, as shown by the k' and α values given in Table II.

Substitution of bromine into the para-position leads to either an increase or a decrease in retention. An increase is observed for prim. and sec. anilines and a decrease for tert. anilines. A similar behaviour was previously noticed for other kinds of alkyilanilines and for

non-alkyl-substituted anilines and its cause discussed in terms of steric hindrance, base strength and solubility in the moving phase¹.

Among the tribrominated meta-alkyl-substituted anilines the retention decreases in the order prim., sec. and tert. anilines, i.e. with increased steric hindrance around the nitrogen atom. The very low k' values of the tribrominated tert. anilines in Table II indicate that these compounds behave on the whole as hydrocarbons on the nitrile phase.

The elution order on the nitrile phase for some meta-alkyl-substituted anilines and their bromination products is further illustrated in Fig. 5. The semi-linear relationship between k' values of brominated and corresponding non-brominated anilines, previously established for the C_{18} phase, is also valid for the nitrile phase (Fig. 6). As can be seen, the position of a bromoaniline is governed by the number of ortho-situated bromine atoms and by the presence of a para-situated bromine atom.

CONCLUSIONS

For the investigated meta-alkyl-substituted anilines the quantitative coulometric bromination method described by Truedsson and Smith² yields up to the end-point mainly o- and p-bromoanilines by exchange of hydrogen for bromine at free ortho- and para-positions. Prim. m-alkylanilines are not particularly sensitive to excess of bromine, unless ortho-situated alkyl groups are also present. In that case, oxidation products appear on over-bromination. Sec. and tert. anilines are not fully brominated at the end-point, but still contain vacant ortho-positions. On over-bromination, these positions are substituted with bromine and, on further bromination, loss of N-alkyl groups occurs with the formation of prim. and sec. anilines.

Introduction of bromine into the ortho- and para-positions causes an increase in retention in the reversed-phase LC system. In the straight-phase system, ortho-bromination occasions a decrease in retention and para-bromination leads to an increase for prim. and sec. anilines and a decrease for tert. anilines.

In both of the LC systems studied, there is a semi-linear relationship between k' values of brominated and non-brominated anilines, respectively.

ACKNOWLEDGEMENT

The experimental assistance of Mrs. Kerstin Svensson is gratefully acknowledged.

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TABLE I

CAPACITY FACTORS, k' , AND SEPARATION FACTORS, α , FOR META-ALKYL-SUBSTITUTED ANILINES AND THEIR BROMINATION PRODUCTS IN THE REVERSED-PHASE LC SYSTEMColumn: Nucleosil C₁₈. Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0).

No. Aniline (Substituent)	k'		α					
	Non-brominated	Monobrominated Ortho	Para	Dibrominated 2,6- 2,4- 4,6-	Tri-brominated	Mono-/non- Ortho Para	Di-/mono-para- 2,4- 4,6-	Tri-/di- 2,4- 4,6-
1 3-Methyl	0.51		0.88	2.17 2.17 5.14		1.73 2.47 2.47 2.37 2.37		
2 3-Ethyl	0.67	1.31	1.12	2.57 2.73 6.24	1.96	1.67 2.29 2.44 2.43 2.29		
3 2,3-Dimethyl	0.74		1.25	3.23		1.69	2.58	
4 2,5-Dimethyl	0.75		1.29	3.26		1.72	2.53	
5 3,4-Dimethyl	0.69	1.37		3.14		1.99		
6 N-Methyl-3-methyl	0.89		1.66	4.33 4.24 7.18		1.87 2.61 2.55 1.66 1.69		
7 N-Ethyl-3-methyl	1.14		2.11	6.55 6.30 10.3		1.85 3.10 2.99 1.57 1.63		
8 N,N-Dimethyl-3-methyl	1.82		3.42	5.18 17.7		1.88	1.51	3.42
9 N,N-Diethyl-3-methyl	3.08		5.83	10.1 31.9		1.89	1.73	3.16

TABLE II

CAPACITY FACTORS, k' , AND SEPARATION FACTORS, α , FOR META-ALKYL-SUBSTITUTED ANILINES AND THEIR BROMINATION PRODUCTS IN THE STRAIGHT-PHASE LC SYSTEM

Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane.

No.	Aniline (Substituent)	k'	α						
			Non- brominated	Monobrominated Ortho Para	Dibrominated 2,6- 2,4- 4,6-	Tri- brominated	Mono-/non- Ortho Para	Di-/mono-para- 2,4- 4,6-	Tri-/di- 2,4- 4,6-
1	3-Methyl	8.07		9.62	3.06	3.06 0.68	1.19	0.32	0.22 0.22
2	3-Ethyl	7.02		1.93 8.31	2.93	2.57 0.60	0.27	1.18 0.35	0.20 0.23
3	2,3-Dimethyl	5.48		7.15		1.88	1.30	0.26	
4	2,5-Dimethyl	4.45		5.50		1.57	1.24	0.29	
5	3,4-Dimethyl	9.51		2.24	0.60		0.24		
6	N-Methyl-3-methyl	2.16		2.62	0.66	0.73 0.37	1.21	0.25	0.56 0.51
7	N-Ethyl-3-methyl	1.40		1.65	0.42	0.45 0.28	1.18	0.25	0.67 0.62
8	N,N-Dimethyl-3-methyl	0.68		0.42		0.37 0.11	0.62	0.88	0.30
9	N,N-Diethyl-3-methyl	0.54		0.42		0.21 0.08	0.78	0.50	0.38

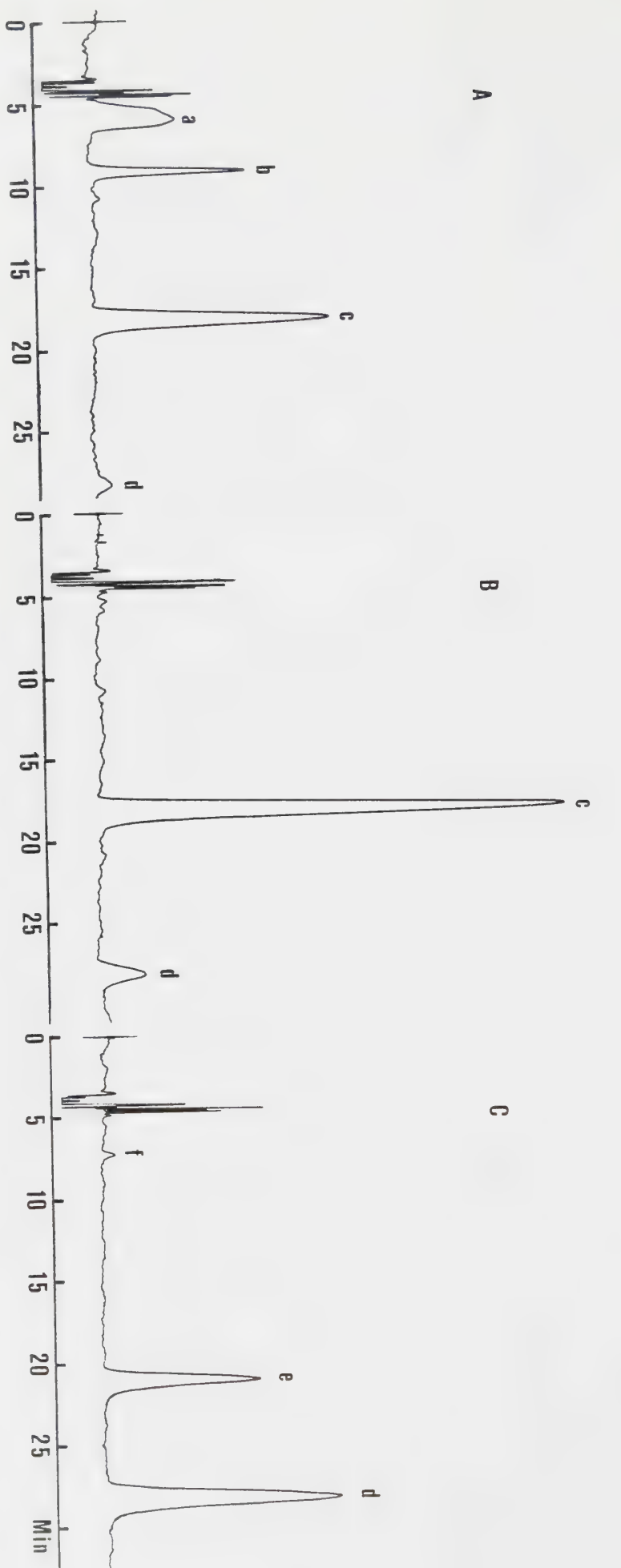


Fig. 1. Chromatograms of the product mixture after bromination of N-methyl-3-methylaniline. Column: Nucleosil C₁₈. Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml · h⁻¹. Wavelength: 280 nm. Volume injected: 20 µl. Peaks: a = N-methyl-3-methylaniline; b = N-methyl-3-methyl-4-bromoaniline; c = N-methyl-3-methyl-2,4-dibromoaniline + N-methyl-3-methyl-4,6-dibromoaniline; d = N-methyl-3-methyl-2,4,6-tribromoaniline; e = 3-methyl-2,4,6-tribromoaniline; f = oxidation product. A, B and C refer to points at which samples were taken (see text).

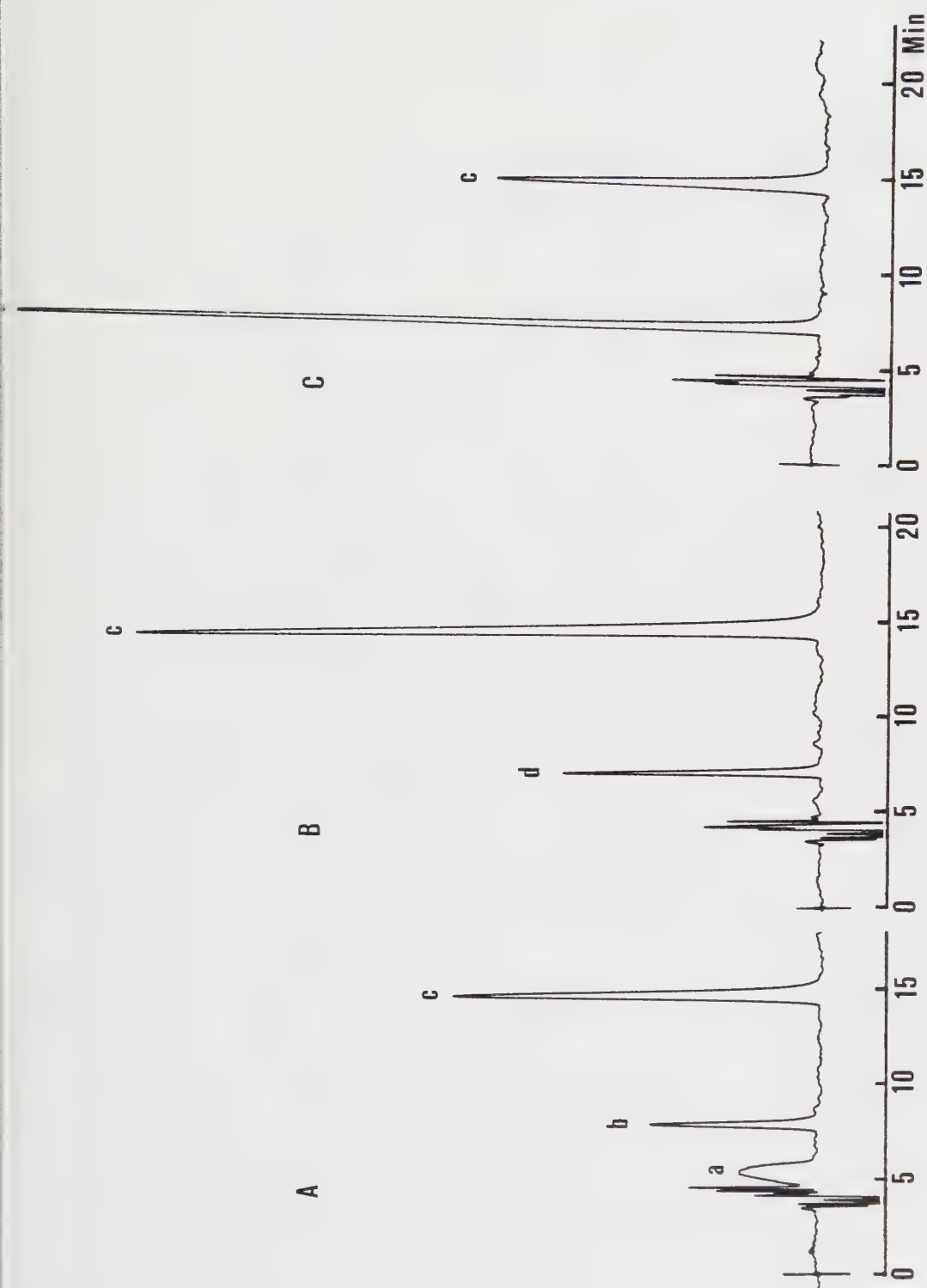


Fig. 2. Chromatograms of the product mixture after bromination of 2,5-dimethylaniline. Column: Nucleosil C_{18} . Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml $\cdot$ h $^{-1}$. Wavelength: 280 nm. Volume injected: 20 μ l. Peaks: a = 2,5-dimethylaniline; b = 2,5-dimethyl-4-bromoaniline; c = 2,5-dimethyl-4,6-dibromoaniline; d = oxidation product. A, B and C refer to points at which samples were taken (see text).

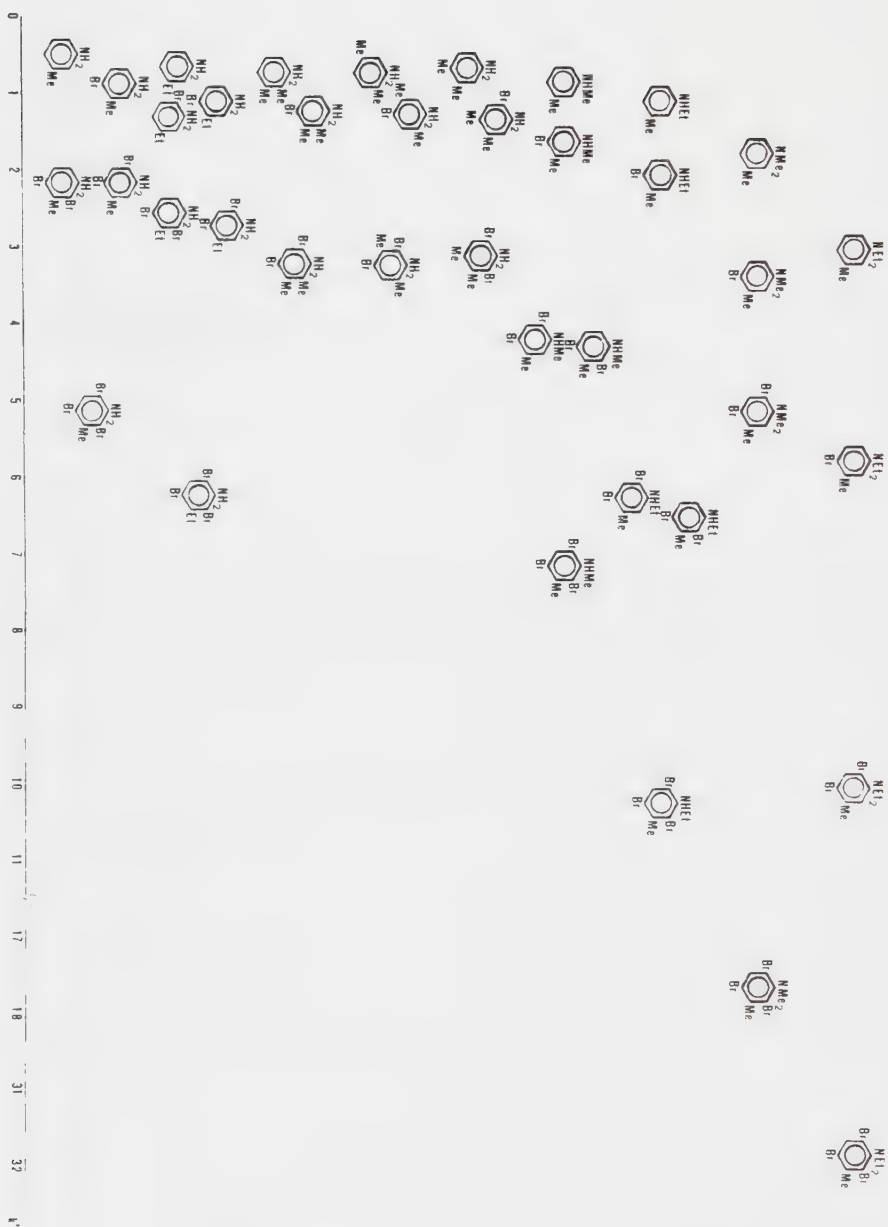


Fig. 3. Retention of prim., sec. and tert. anilines and the corresponding bromination products in reversed-phase LC. Column: Nucleosil C₁₈. Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml · h⁻¹.

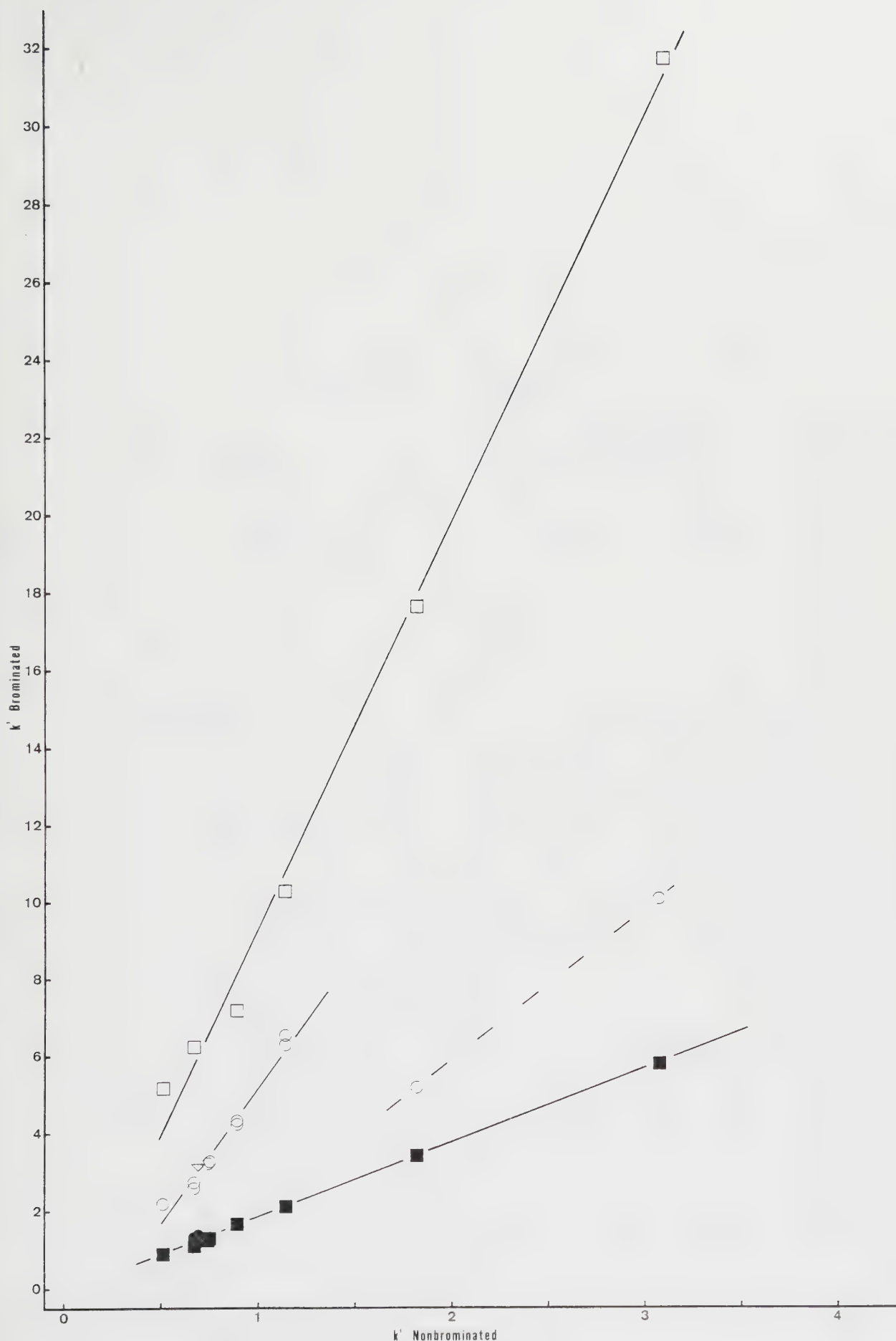
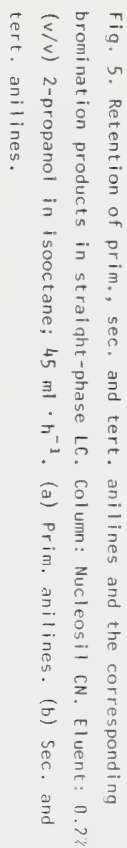


Fig. 4. Relationship between capacity factor, k' , for alkyranilines and the corresponding bromination products in reversed-phase LC. Column: Nucleosil C₁₈. Eluent: methanol-aqueous buffer (80:20, v/v, pH = 7.0); 45 ml · h⁻¹. ■ = mono-para-brominated; ● = mono-ortho-brominated; ○ = ortho-para-brominated; ∇ = di-ortho-brominated; □ = tribrominated.



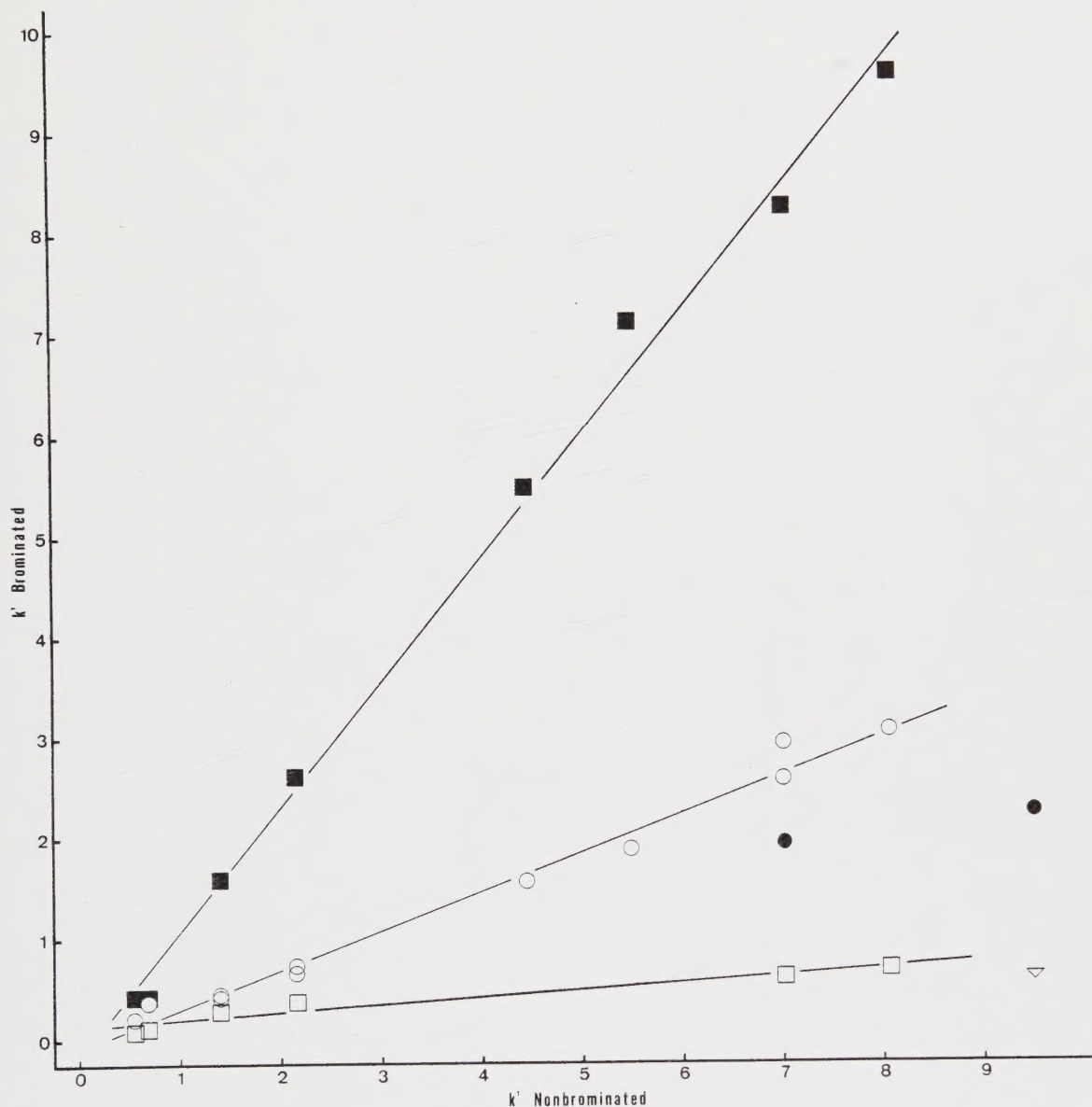


Fig. 6. Relationship between capacity factor, k' , for alkyranilines and the corresponding bromination products in straight-phase LC. Column: Nucleosil CN. Eluent: 0.2% (v/v) 2-propanol in isooctane; $45 \text{ ml} \cdot \text{h}^{-1}$. ■ = mono-para-brominated; ● = mono-ortho-brominated; ○ = ortho-para-brominated; ▽ = di-ortho-brominated; □ = tribrominated.

